Joseph Gilala

DETERMINATION OF PHENOLS IN WATER BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH A UV-DETECTOR

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Laboratorium Badania Wody i Gleby, Wojewödzkiej Stacji Sanitarno-Epidemiologicznej Poznan, Poland

Water and soil testing laboratory of regional sanitary and epidemiological station in Poznan, 61-705 Poznan ul. Noskowskiego 21

Tel. (0-61) 8544–829, 8544–829 fax. (0-61) 8544–829

www.wssepoznan.pis.gov.pl

E-mail: lbwig@ wssepoznan.pl

Host supervisor: Jarosław Tokarski

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THESIS ABSTRACT

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This study is based on the SPE process, and the method employed was validated on 11 compounds of phenol using phenol calibration mix. It focuses on the determination of phenols in water by high performance liquid chromatography with a UV-detector.

The background section deals with phenols, its subsequent group, the analytical methods and laboratory equipments used in determining these compounds in water. The experimental section provides information on the validation and optimization of analytical method according to the ISO standard or EPA method concentrates on specific group of phenols.

High performance liquid chromatography with a UV-detector is the main analytical equipment used in determination of phenols in water. The water samples to be analyzed were taken from five different locations in Poznan, Poland. The experiments were done in the water and soil testing laboratory of regional sanitary and epidemiological station in Poznan, Poland. The objective of the developed method was to achieve a concentration of 0.1 mg/l of each phenol from water samples and excellent recovery between 80-110% based on the column employed.

In conclusion, with the method developed, it can be prove that phenols were not present in water within the working range of 0.003-0.250 mg/l.

Key words: High performance liquid chromatography, ultraviolet detector, solid phase extraction, water analysis, phenols

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1 INTRODUCTION

The human race is influenced by the quality of water resources in the natural environment. In order to guarantee a secure and healthy life, the water consumed by everyone must be free from harmful chemical substances. The water released into the environment by water purification plants and private organizations should be regularly accessed and examined to maintain complete yielding to water framework act. Thus, treated waters should be safe for consumption and free from harmful compounds to guarantee a healthy life.

However, there are environmental agencies for water which have given standards to ensure that water is free from contaminants, thus making safe water consumption possible. Water treatment requires adequate analysis and monitoring which includes determination of low level contaminants. The maximum level of contaminants present in drinking water should be so small that the effect is harmless to human life. Thus analytical methods are important to quantify the contaminants.

Phenol and nitro-phenols are natural harmful substances found in water. They are used in many industrial activities for the production of pesticides, insecticides, herbicides, and synthetic products. Phenols which contain certain amount of pesticides and wood preservatives can leads to specific health damages even at the lowest concentration levels. Nevertheless, it is important to be aware of phenols and substituted phenols in environmental and biological samples. Substituted phenols can be named using suitable prefixes which are the ortho- (1, 2), meta- (1, 3) and para- (1, 4) system depending on the placement of the substituent from the hydroxyl group. Liquid chromatography with a UV detector is one analytical approach used due to its high selectivity for phenols. However, methods have been developed for the determination of phenol by gas chromatography with a flame ionization detector, which is quite effective but this research will concern a different method for determining the eleven possible phenols as listed in the U.S.EPA pollutant guideline.

Solid phase extraction is a known method used for sample preparation techniques. It is the most recognized technique for aqueous sample extraction, sample cleanup and concentration. This method is applied to my research. Method detection limits of liquid

chromatography techniques for direct sample injection in determining low level concentration in natural waters are not easily quantified, and thus it is necessary to carry out sample pre-concentration before analysis using solid phase extraction. Method detection limits can be used to create an environment which enhances a specific compound retention on the chosen solid phase extraction column.

This research study concerns the validation and optimization of an analytical method for phenol determination in water. The aims of this thesis is to determine phenols present in water by high performance liquid chromatography with an ultraviolet detector and, secondly, to test different columns for their retention property of analytes as well as other matrixes.

The objective of this study is to develop a method with high recovery rate under various conditions and to estimate the performance characteristics of an analytical procedure including limit of detection and quantification, accuracy, recovery, precision, trueness of an analytical method and uncertainty. This research work is limited to phenols and phenolic compounds in water, method development and verification.

The research questions concerning this study are the following: Can the developed method be applied and is it a trusted method in determining phenols in water? Is it fit for purpose? Does the developed method pass the requirement set by the EPA guidelines? The purpose of the developed method is to achieve a concentration of 0.1 mg/l of each phenol from water samples and excellent recovery base on the column employed.

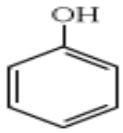
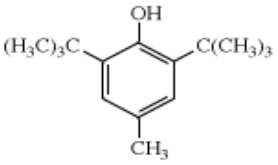
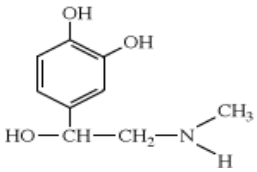
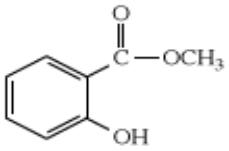
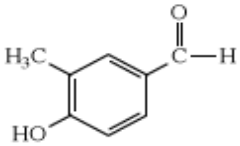
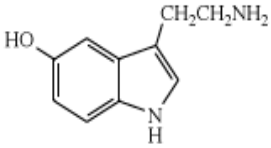
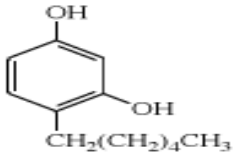
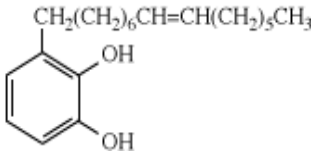
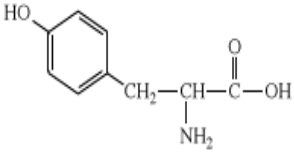
The water samples used for the research work are from five different locations in Poznan: Uli Lake ($52^{\circ}\ 25'\ 38''$; $17^{\circ}\ 22'\ 04''$), Baba Lake ($52^{\circ}\ 25'\ 14''$; $17^{\circ}\ 21'\ 57''$), Dobre Lake ($52^{\circ}\ 28'\ 19''$; $17^{\circ}\ 17'\ 41''$), Debiniec Lake ($52^{\circ}\ 27'\ 59''$; $17^{\circ}\ 13'\ 46''$) and Malta Lake. Thus, every sample is to be analyzed twice for accuracy.

The experiments are to be performed in water and soil testing laboratory, Poznan, Poland. High performance liquid chromatography is the main analytical method for detecting phenols in water with UV-detector. The main part of this study discusses the analytical instruments used in the research, the method development, and phenols.

2 THEORETICAL FRAMWORK

Phenols are classified as organic compounds similar to alcohols, but they form stronger hydrogen bonds. They are characterized by the hydroxyl (-OH) group which is attached to a carbon atom and is part of an aromatic ring. Phenols which have a structural formula (C_6H_5OH), that is the formula of phenol only, as a simplest member of phenols, and subsequent phenols with structure R- C_6H_4OH, where R represents some groups like CH_3, and NO_2. However, phenols are more soluble in water than alcohols and possess a higher boiling point. Phenols are highly toxic colorless liquids or white solid at room temperature. (Wade 1999.)

TABLE 1. The structural formula of some groups of phenol compounds (Wade 1999)

 phenol	 2,6-di-t-butyl-4-methyphenol	 epinephrine(adrenalin)
 methylsalicylate	 vanillin	 serotonin
 4-hexylresorcinol	 urushiol	 tyrosine

However, some commonly used names of certain phenolic compounds are vanillin, salicylic acid, pyrocatechol, resorcinol, cresol, hydroquinone, and eugenol. Hence, phenol having one substituent can be classified with the right number or the ortho (1,2), meta (1,3) and para (1,4) pattern (Wade 1999).

TABLE 2.Structural formula of substituent phenols (Wade 1999)

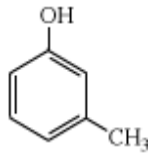
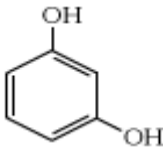
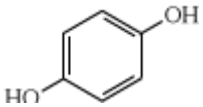
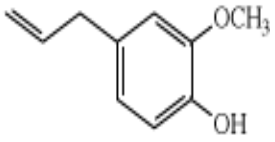
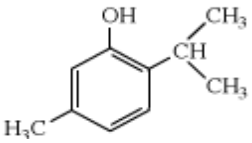
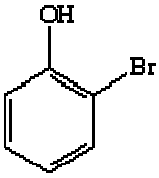
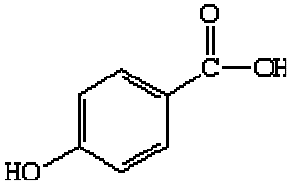
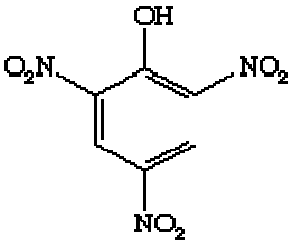
 meta-cresol	 resorcinol	 hydroquinone
 eugenol	 thymol	 2-bromophenol (ortho-comopund)
 4-hydroxybenzoic acid (para-compound)	 2,4,6-trinitrophenol	

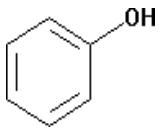
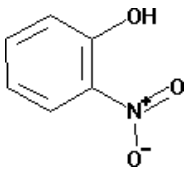
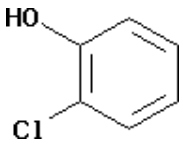
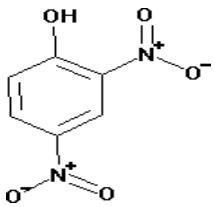
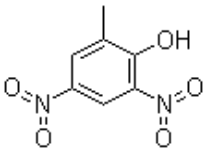
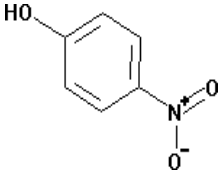
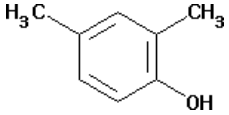
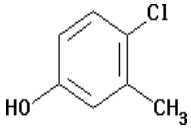
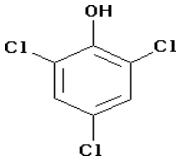
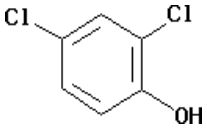
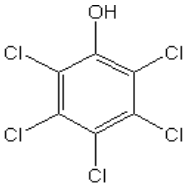
Table 3 gives an overview of phenolic compounds, their parent compounds and possible uses in the environment.

TABLE 3. Phenolic compounds, their parent compounds and examples of environmental sources (BCER COTC Fact sheet on phenols 2007)

	Chemical name	**Abbreviation**	**Parent compound if applicable**	**Additives, commercial & personal product exposure sources**
1	Bisphenol A			Polycarbonate containers and coatings (cans, cups), dental sealant
2	Benzophenone-3(2-hydroxy-4-methoxy-benzophenone), (oxybenzone)	BP3		Sunscreen agent, photostabilizer for synthetic resins
3,4,5	2,4-dichlorophenol and trichlorophenols (chlorinated phenols)	24DCP, 245TCP, 246TCP	Phenoxy- and other derivatives(245,246 TCP are metabolites of hexachlorobenzene and hexachlorocyclohexane)	Herbicides (organochlorine pesticides)
6	2,5-dichlorophenol	25DCP	4-dichlorobenzene (metabolite of p-DCB)	Mothballs
7	ortho-phenylphenol	o-PP		Fungicide
8	4-tert-octylphenol	4-t-OP		Detergent surfactant
9	Triclosan[5-chloro-2-(2,4-dichlorophenoxy)phenol]	TRCS		Microbicide in home cleaning and personal care products

Table 4 illustrates the eleven structural formulas of phenols stated by some environmental agency and they are the lists of phenols to be determined in water analysis.

TABLE 4. Structures of the eleven phenols specified in the U.S.EPA priority pollutants list (Dionex Corporation 2008)

 Phenol	 2-nitrophenol	 2-chlorophenol	 2,4-dinitrophenol
 2-methyl-4,6 dinitrophenol	 4-nitrophenol	 2,4-dimethylphenol	 4-chloro-3-methylphenol
 2,4,6-trichlorophenol	 2,4-dichlorophenol	 Pentachlorophenol	

2.1 Sources of phenols

Phenols mainly occur in nature as a product of coal tar or crude petroleum. However, phenols are formed as natural decay of organic compounds. Phenols present in nature include tyrosine found in proteins which belong to the class of amino acid. Epinephrine is an adrenaline that produces hormone. Serotonin is a neurotransmitter found in the brain

and urushiol, which causes irritation generated by poison ivy to prevent animals from doing certain things. Some phenol can be gotten from plant like thymol, separated from thyme and eugenol, extracted from cloves. (Wade 2009.)

2.2 Uses of phenols

Phenol is used as raw material in the manufacture of a wide range of important chemicals including phenolic resins, bisphenol-A, caprolactam, alkylphenols and adipic acid. Phenol is widely used for the treatment of injuries. It is suitable for making aspirin drug also antiseptics and local anaesthetics (Wade 2009).

Phenol is used in the manufacture of paints and varnish removers, lacquers, rubber, ink, and illuminating gases, tanning dyes, perfumes, toys and soaps (Wade 2009). Phenol is used as an industrial chemical in the manufacturing of certain products such as “resins, plastics, fibers, adhesives, iron, steel, aluminum, leather, and rubber”, also phenol is present in disinfectants, cigarette smoke, and emissions from vehicles (EHC 161, 1994).

Phenols have a wide range of applications in household products and industrial synthesis. They are used as disinfectants in household cleaners, lotions, salves, ointments and in mouth wash. However, compounds of phenols are used in dye industries to make colored azo dyes and used as components in making wood preservatives such as creosote. Industrial applications of phenols are used in making plastics, explosives like picric acid, and drugs such as aspirin. (Wade 1999.)

2.3 Physical properties of phenols

Phenols which are identical to alcohols having a hydroxyl group attach to an aromatic ring which enables them to undergo intermolecular hydrogen bonding. They have the ability to form stronger hydrogen bond than alcohols. Nevertheless, the presence of hydrogen bonds in phenols makes them to be more soluble in water. Hence, the occurrence of hydrogen bonds in phenols results in higher melting and boiling points. (Wade 2009.)

Table 5 summarizes the physical and chemical data of eleven phenols specified by certain environmental agencies in their priority pollutants lists. The eleven groups of phenols are determined in water to ensure that the concentration does not exceed the limit provided by the environmental agencies.

TABLE 5. Physical and chemical data of Phenols (Merck chemicals 2010)

	Formula	**Molar mass (g/mol)**	**Melting point (℃)**	**Boiling point (^{o}C)**	**Density (g/cm^{3})**
Phenol	C_6H_5OH	94.11	40.8	181.8	1.06 (20 ℃)
2-nitrophenol	$2\text{-}(NO_2)C_6H_4OH$	139.11	43 - 45	215 - 216	1.26 (20 ℃)
2-chlorophenol	$2\text{-}(Cl)C_6H_4OH$	128.55	7	174	1.26 g (20 ℃)
2,4-dinitrophenol	2,4-DNP	184.11	114 - 115		1.68 (20 ℃)
2-methyl-4,6-dinitrophenol	$C_7H_6N_2O_5$	198.14	82 - 85	312	
4-nitrophenol	$O_2NC_6H_4OH$	139.11	110 - 115	279	1.48 (20 ℃)
2,4-dimethylphenol	$2,4\text{-}(CH_3)_2C_6H_3OH$	122.16	25	211	1.016 (25 ℃)
4-chloro-3-methylphenol	$4\text{-}(Cl)\text{-}3\text{-}(CH_3)C_6H_3OH$	142.58	63 - 65	235 - 239	1.37 (20 ℃)
2,4,6-trichlorophenol	$C_6H_3Cl_3O$	197.44	65 - 68	244 - 246	1.675 (25 ℃)
2,4-dichlorophenol	$2,4\text{-}(Cl)_2C_6H_3OH$	163	40 - 43	209 - 211	
Pentachlorophenol	C_6HCl_5O	266.34	190-191	309-310	1.978 (22 ℃)

3 METHODS FOR DETERMINING OF PHENOLS IN WATER

There are methods which have been developed over the years for the determination of phenolic compounds in water and waste water. Some of these methods are spectrophotometry, electrochemical methods, capillary electrophoresis, the gas chromatographic (GC) method using liquid-liquid extraction and either using flame ionization detection (FID) or derivatization and electron capture detection (ECD) to analyze different phenols at a low concentration. In determination of phenol at high concentration, the gas chromatography/mass spectrometric (GC/MS) method with liquid-liquid extraction is employed. (Eaton, Clesceri, Rice & Greenberg 2005, 79-80.)

In water treatment plant, chlorine applications have resulted in producing chlorophenol. The method which can be applied in analyzing phenol, ortho- and meta- substituted phenols is known as 4-aminoantipyrine colorimetric method. However, para-substituted phenol with sub group known as carboxyl, halogen, methoxyl or sulfonic acid group cannot be determine using 4-aminoantipyrine method under certain pH ranges. Thus, 4-aminoantipyrine method is suitable for water samples with high sensitivity. The disadvantage of this method is that any color produced by the reaction of any phenolic compounds is proved to be phenol. (Eaton et al. 2005, 43-44.)

Chemiluminescence (CL) is an analytical detection method suitable for very low detection limit, fast and large linear working range that can be obtained using simple instrumentation. Chemiluminescence method is applied for the determination of phenol, including luminal CL system and acidic $KMnO_4$CL system. However, due to lack of selectivity for phenol, chemiluminescence systems cannot determine phenol in water samples directly. Phenol can be determined only when the CL system is combined with some separation precession like per-distillation, liquid chromatography and capillary chromatography. (Huili, Jiagen, & Baoxin 2006.)

Liquid-liquid extraction gas chromatographic method is applied in analyzing phenols and some substituted phenols in water either in municipal or industrial released. During confirmation of an unknown compound, the use of derivatization, cleanup and electron capture detector gas chromatography (ECD/GC) are used to determine results obtained by

flame ionization detector gas chromatographic (FID/GC) method. (Eaton et al. 2005, 79-80.)

Method 8041 gives a wide number of options for the determination of phenols in water and soil samples. Phenols are separated from water at pH < 2 with methylene chloride using liquid-liquid or continuous liquid extraction. Phenols is analyzed by FID using one column or double column procedure after solvent evaporation and replacing the solvent to 2-propanol. Thus, sensitivity may not be suitable for the underivatized phenols. Phenols can be derivatized with diazomethane to produce methyl ester of phenol and can be determined by FID. A suitable approach for sensitivity and selectivity can be achieved by derivatizing the analyte extracts with pentafluorobenzylbromide (PFBBr) and detecting the derivatized phenols using electron capture detector (ECD). Hence, three phenols: 2, 4-dinitrophenol, 2-methyl-4, 6-dinitrophenol and dinoseb are not derivatized by PFBBr. During cleanup process, a silica gel is used after the derivatization. (Grob & Barry 2004.)

Another method for derivatization is the use of acetic anhydride ($C_4H_6O_3$). The sample is adjusted to pH≈7 using sodium hydroxide or phosphoric acid and adding potassium carbonate and acetic anhydride to the sample. After mixing, hexane is used to extract the derivatives then the extract is injected into gas chromatography column. This method is not suitable for nitrophenols because of the poor effectiveness of the derivatization reaction. (Dmitruk, Zbiec & Dojlido 2006.)

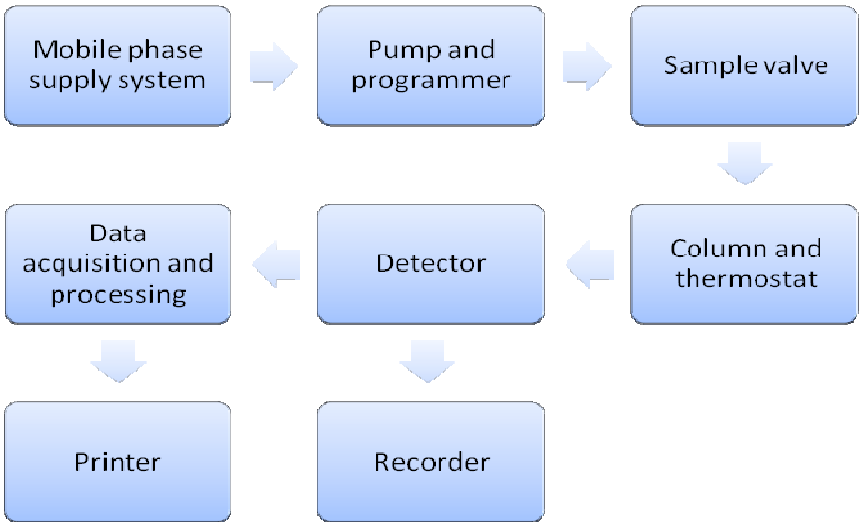
The characteristics of high performance liquid chromatography (HPLC) are proved to be an effective method for the separation of phenols. However, improvements have been made in HPLC analysis of compounds. In recent years, devices for detection and identification coupled to HPLC have been developed to make separation, qualification and quantification of compounds possible. HPLC methods for analysis of compound avoid the difficulties and time-consuming separation of compounds for the subsequent individual identification of each compound. Thus, in this research, information of the analysis of phenols by HPLC is provided.

4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In our present time, there is a rising interest in applying high performance liquid chromatography (HPLC) which is not subject to temperature dependence to the determination of not just volatile organic compounds like aliphatic and polyaromatic hydrocarbons, saturated and unsaturated aliphatic halogen compounds, haloforms and some esters, phenols unlike the gas chromatography, but for all organic and inorganic matter present in water samples. Hence, in liquid chromatography, a liquid passes through a porous solid stationary phase and the elute flows through a detector. In HPLC, the mobile phase is pumped at high pressure (Crompton 1999, 57.)

The essential parts of liquid chromatography include the mobile phase of solvent, high pressure and low pressure gradient programmers, pumps (piston and diaphragm pumps, syringe and rapid refill pumps), valves and oven column. The detectors used in high performance liquid chromatography include UV detector which can be fixed or variable wavelength, the fluorescence detector and the refractive index detector. An HPLC stationary phase includes irregular and spherical silica gel. (Scott 2008.)

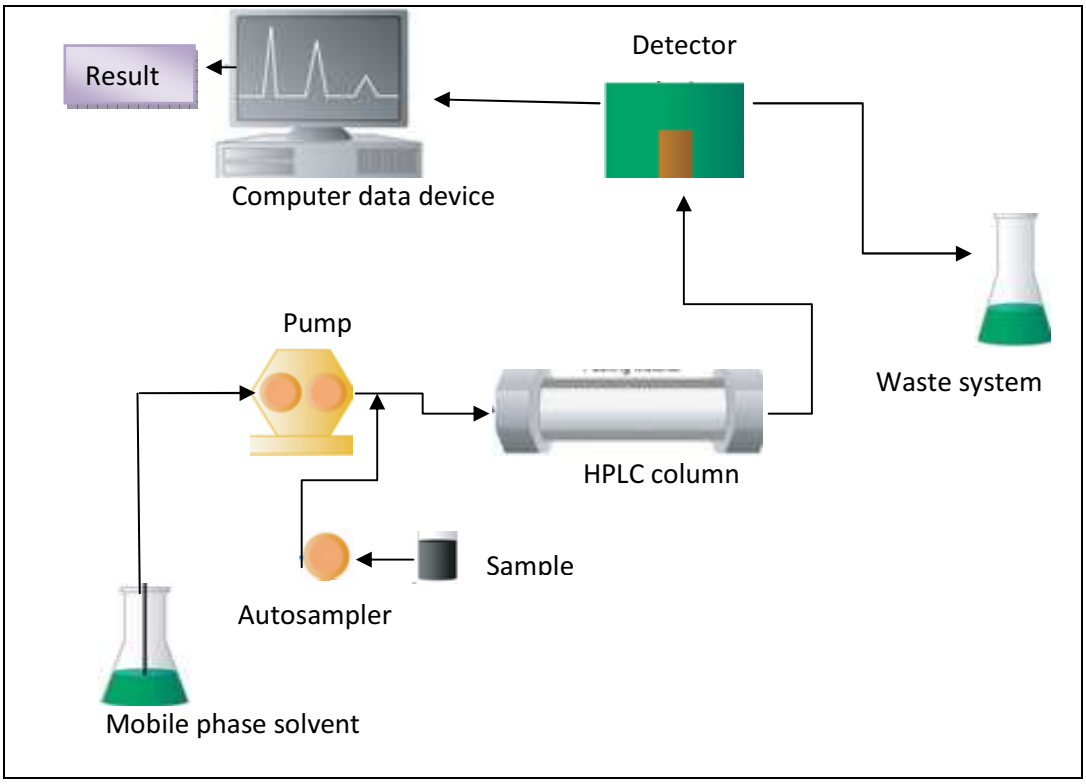
Graph 1 gives a clear illustration of the essential parts of liquid chromatography from one component part to another.



GRAPH 1**.** The essential parts of liquid chromatography copyright by Scott 2008

The mobile phase which is a solvent is contained in a bottle. A high pressure pump is needed to deliver the mobile phase at a certain flow rate apparently in milliliters per minute. Also, an injector known as auto-sampler passes samples into a constant moving mobile phase stream that moves the sample into the HPLC column. The stationary phase is a chromatographic material packed in a column and it is needed to effectively carry out the sample separation. Furthermore, a detector helps to visualize the separated sample bands eluting the HPLC column and the mobile phase leaves the detector which is collected by waste system. However, the detector is connected to a data collection system that stores the electrical signal that produces the chromatogram on its screen. The end results are seen as chromatogram appearing as peaks of various heights depending on the concentration of the sample constituents. (Waters Corporation 2010.)

Graph 2 gives a concise picture on the operation of high performance liquid chromatography (HPLC) system.



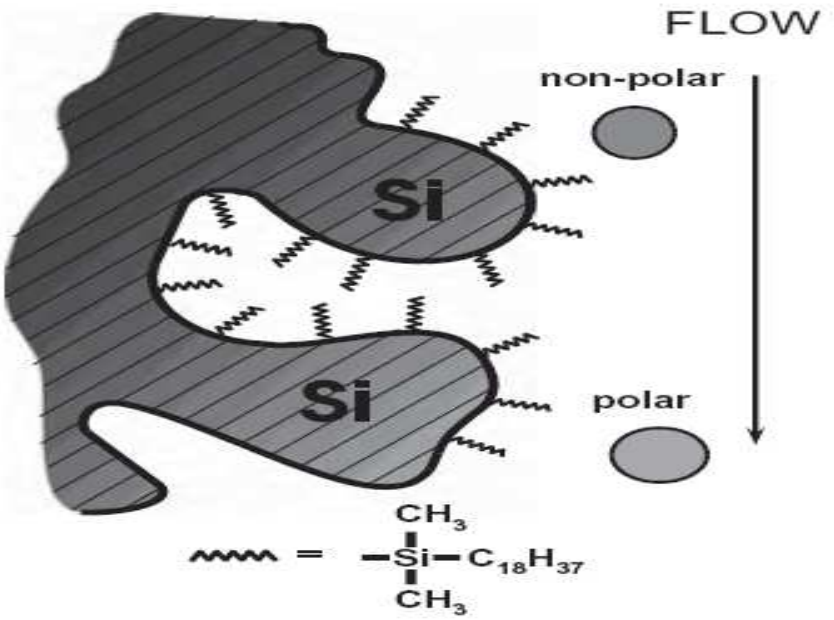
GRAPH 2. Operation of HPLC system copyright by Waters Corporation 2010

4.1 Types of chromatography column

High performance liquid chromatography (HPLC) have been created so as it can perform to a very high level by combining selective stationary phases of different material sizes with it, also with adequate columns with big amount of plates per liter. There are different types of chromatography column used in high performance liquid chromatography which are reversed-phase chromatography, reversed-phase ion-pairing chromatography, ion-suspension chromatography and ion-exclusion chromatography. Reversed phase chromatography (RPC) column are mostly used in all HPLC applications. (Crompton 1999, 57.)

4.1.1 Reversed phase chromatography

This is the most commonly used chromatographic mode in HPLC. It is used for the analysis of wide range of neutral compound which are carbohydrates and polar organic compounds. Reversed phase chromatography is mostly performed by using bonded silica-based columns by limiting the pH range to 2.0 - 7.5. (Crompton1999, 57.)



GRAPH 3. Reversed-phase chromatography column copyright by Dong 2010

5 LIQUID CHROMATOGRAPHY DETECTOR

In choosing the right kind of detector in liquid chromatography, it is highly important to consider the selectivity, response, sound, specific values and linearity (Scott 2008). The different detectors that can be used in analytical procedures include some refractive index equipments, detectors associated with an ultraviolet absorption, also fluorescence detection. These detectors are widely used in LC applications because of its sensitivities and linearity (Scott 2008).

5.1 Ultraviolet (UV) detector

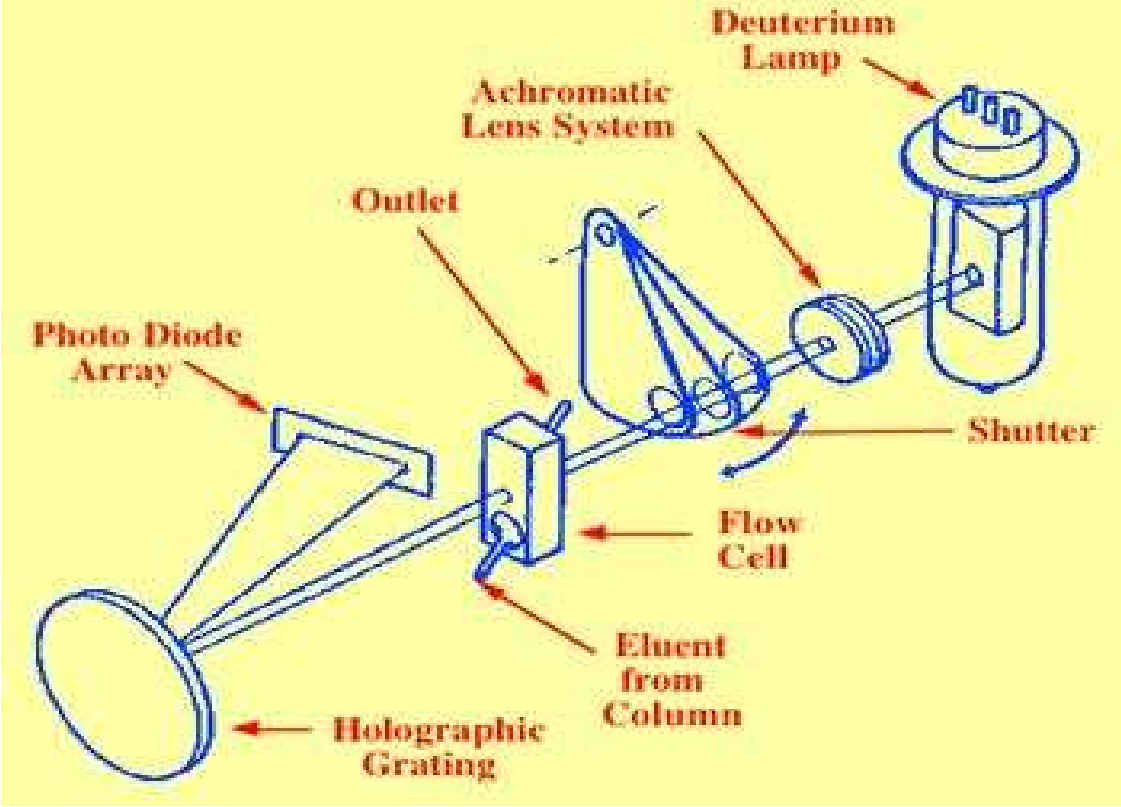
The UV detector is well known and widely employed in all LC applications and there are some difficulties associated to this detector when used for analyzing non polar compounds that lack UV chromaphores. Nevertheless, ultraviolet (UV) detector has good selectivity, linearity, versatility and reliability for separation of analytes. In recent times, different LC detectors have been developed for different purposes. Hence, the different types of UV detector which can be used are the diode array detector, fixed wavelength detector and the multi-length wavelength detector. (Scott 2008.)

Diode array detector uses deuterium or xenon lamp which emits light over the UV spectrum range. Light from the lamp is shown by an achromatic lens through the sample cell and onto a holographic grating. The separated light from the grating falls on a linear diode array. The resolution of the detector (Δy) depends on the number of diodes (n) in the array, also on the range of wavelengths covered ($\lambda 2 - \lambda 1$) which can be illustrated by the equation as follows;

$$\Delta y = \frac{\lambda 2 - \lambda 1}{n}$$

Nevertheless, the resolution power of the diode array depends on the semi-conductor and on how narrow the individual photo cells can be constructed. (Scott 2008.)

Graph 4 gives a clear picture of the operation diode array detector. Light rays from a deuterium lamp moves parallel by an achromatic lens to focus it through a detector cell on a holographic grating and the sample is exposed to it at all wavelength produced by the lamp. However, the light generated from the grating falls onto a diode array. The array consists of many diodes and the output from a single diode is sampled by a data system which is stored on a hard disc. The output from the diode is selected and a chromatogram is produced by UV wavelength falling on the diode. (Scott 2008.)

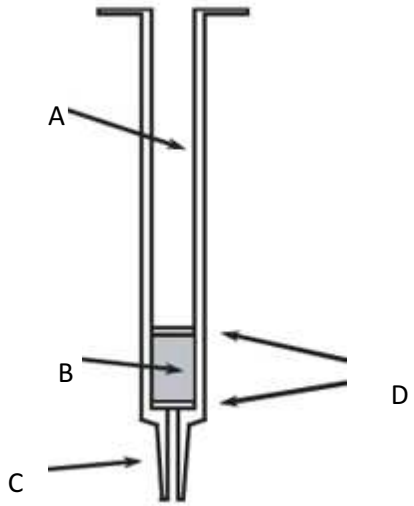


GRAPH 4. The diode array detector copyright by Scott 2010

6 INTRODUCTION TO SOLID PHASES EXTRACTION

Solid phase extraction is a method widely applied to separate and purify analyte from the sample. In analytical technique, solid phase extraction is a useful procedure employed for separating analyte from its matrix also for sample purification and enrichment. It is applied to hold analytes on a sorbent, derivatized before it is extracted from the solvent. (Biotage 2008.)

Unlike liquid-liquid extraction, solid phase extraction is an easy method and have great advantages in concentration of analytes, high recovery rate of analytes, and it is more compatible in analytical analysis. Solid phase extraction uses a separation column that is easily disposed after performing an experiment. Graph 8 shows a typical solid phase extraction column and its component parts with the labeled A as reservoibr, B as sorbent bed, C known as luer tip and D as frits which made of polyethylene stainless steel or teflon. (Biotage 2008.)



GRAPH 5. Solid phase extraction column copyright by Biotage 2008

The procedure in applying solid phase extraction involves passing the water sample into SPE column where the analytes are eluted and absorbed on the separation column.

However, using the right elution solvent can ease the removal of obstacle from the column. Above all, the required analytes is gotten from the SPE column using elution solvent will result into more purified product. Thus, the process increases the eluted analytes concentration than in the initial sample. Selecting an efficient separation column that could prevent unwanted substances in the sample, also enable desired product to flow through without been retained on the column. (Biotage 2008.)

6.1 Columns in solid phase extraction

Solid phase extraction (SPE) columns are present in different kinds of sorbent to be used in analytical techniques which result to the presence of its outstanding retention capability and selectivity. The intermolecular interaction that exist in SPE includes polar bond due to the presence of hydrogen bond or dipole-dipole bond, non-polar bond which is van der Waal attractions, ion exchange among anions and cations and the mixed interaction between ion exchange and non-polar bond. (Biotage 2008.)

6.1.1 Sorbent selectivity

The great selectivity of sorbent in SPE has a significant role in sample extraction. Sorbent selectivity is a process whereby an extraction method is used to separate analyte from obstacles from the initial sample. The degree of selectivity results to the outstanding ability to retain materials, to address large area of analyte characteristics. In contrast to liquid-liquid extraction, the two phases cannot mix together which means that an aqueous sample is not possible to be extracted with methanol. However, in SPE one phase is a solid sorbent which is immiscible with any used extraction solvent and thereby promoting sorbent-solvent interactions with the ability to have high selective extractions. (Biotage 2008.)

6.1.2 Silica based sorbents

In SPE approach, “ISOLUTE sorbents” are categorized based on their retention interaction which are non-polar, ion exchange, mixed mode and polar. Thus, due to “ISOLUTE properties”, it has a way of retaining analytes due to the interconnectivity between analytes and sorbent. The mechanism between analytes and sorbent includes hydrogen bonding and dipole-dipole forces (polar interactions), van der Waal forces in non polar interactions and cation-anion interactions present in ionic interactions. (Biotage 2008.)

Non-polar SPE columns: The non-polar SPE column is used to remove organic compounds from aqueous matrixes. It provides a medium for interaction which makes it easy to extract basic compounds and polar analytes from aqueous matrices. It is better to screen different sorbents and take the one that gives better recoveries and cleanliness. (Biotage 2008.)

Polar SPE columns are used to remove desired sample with polar groups from non-polar matrices. Polar interaction exists among dipole-dipole, dipole-induced dipole and hydrogen bonding due to their different selectivity to enable effective separation as a result of their difference in structural isomer. (Biotage 2008.)

Mixed-mode SPE columns: In mixed mode SPE columns, the double retention interactions is supplied by non-polar and cation exchange mechanism which gives the possibility of high extraction cleanliness. It is mostly used in extraction of drugs from biological fluids. (Biotage 2008.)

The ion exchange solid phase extraction column comprises both weak-strong anion and cation exchange sorbent which make it suitable to extract compounds having a positive or negative charge from both aqueous and non-aqueous solutions. (Biotage 2008.)

7 SOLID PHASE EXTRACTION DEVELOPMENT PROCEDURES

The steps in SPE method include sample pre-treatment followed by salvation of column, column balancing before adding the samples into the column, thus column washing is necessary if there is any interference and lastly extracting analytes from the column. The figure below summarizes the necessary steps toward SPE method.

GRAPH 6. Procedure in solid phase extraction copyright by Joseph 2010

Control flow rate of sample application: In sample application, it is important to maintain slow flow rate in order to achieve high recovery of the analyte from the matrix. However,

too fast flow rate can result to low recovery of analytes, or inappropriate elution when removing the analytes from the adsorbent. (Biotage 2008.)

Sample pre-treatment: This is an important stage when working with HPLC and the main purpose of sample experimentation is to provide a condition that enhances sample secretion on a particular column used in the extraction process. Consequently, before the actual experiment, it is mandatory to dilute the sample to decrease the viscosity or applying a buffer solution to adjust the pH of the sample prior to retention by non-polar or ion exchange sorbents before filtration so that impurities does not block the column. (Biotage 2008.)

Salvation of solid phase extraction column: Salvation of solid phase extraction column is the addition of an appropriate solvent to the column to enhance phase interference between the material that absorb the liquid known as sorbent and the sample before sample application. This process create an environment whereby the require substance can act upon the sorbent material by employing some chemicals that will soften the sorbent, thus a specified volume of solvation liquid is need by the sorbent for proper interaction of the medium. (Biotage 2008.)

Column equilibration apparatus: Balancing of the column is needed to meet the requirement of the pre-treated sample which will enhance high analyte recovery after column solvation. Usually, in balancing the column, liquids are added to promote efficient interaction of the mixture. This is done by pH adjustment to provide ionization of the analyte either by adding a buffer solution with appropriate pH and ionic strength. (Biotage 2008.)

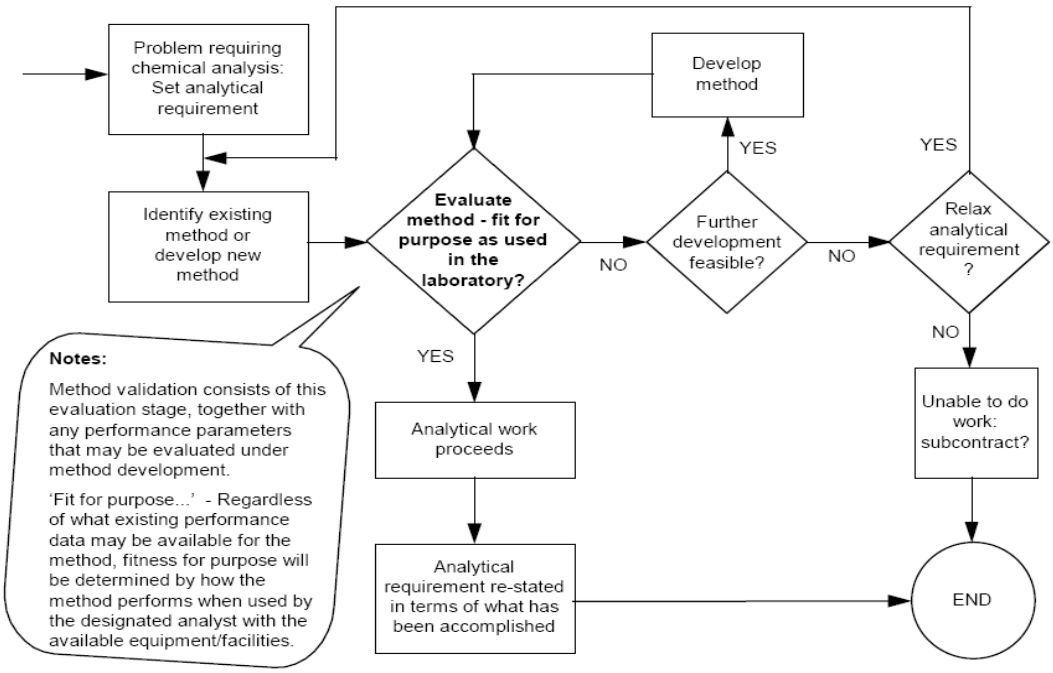
Loading of sample and interference elution: In method development steps, the sample application to the balanced column is depended on the flow of the sample which will determine the extraction efficiency and a specific volume of column. However, a required flow velocity should be maintained. Following sample loading is the interference elution which uses solvent for elution to give maximum analyte recovery and good retention

condition of analytes. It is recommended to keep steady the pH and ionic strength when carrying out elution of interference. (Biotage 2008.)

Analyte elution in sample pre-treatment: The choice of solvent to be used is important in eluting the analyte, Thus it is important that the solvent is able to dissolve the required sample to be analyzed. Nevertheless, the solvent should have affinity for dissolving the analyte to ensure efficient recovery. Hence, method development entails not just knowing the effects of various flow rates, but the condition of analyte elution. (Biotage 2008.)

8 METHOD VALIDATION

Method validation in analytical chemistry is a defining requirement and confirmation that methods under consideration have performance capabilities and it is consistent according to requirement. It is also the process of accessing a method's performance capabilities. In method validation, it is important that the equipments are in the right specification and working range. Method validation has a direct relationship to method development. Thus, method performance parameters are connected with validation method and are often evaluated. The process of assessing the standard of method efficiency and confirming that is reliable for purpose can be seen in the GRAPH 10. However, it gives the necessary questions which need to be considered in an analytical procedure and the performance variables. (EURACHEM Guide 1998.)



GRAPH 7. Method evaluation procedure (EURACHEM Guide 1998)

The analytical requirement for the methods validation are determine by the accuracy of the method, precision of the developed method, limit of detection/limited of quantification, trueness, recovery and uncertainty.

Accuracy: This is the closeness of agreement between results tested and the real value. It is therefore applied to a given set of test results belonging to a random constituent and a common systematic error. (ISO 3534-2, 2006.)

Recovery: A known amount of compound is added to a real sample forming a spiked solution and the two are analyzed, thus the difference in concentrations found being used to calculate the recovery. (ISO/TS 13530, 2009.)

Percentage recovery ($\%f$) is calculated as follows:

$$f = \frac{\bar{C}_z - \bar{C}_p}{\bar{C}_o} \times 100\%$$

$\bar{C}z$= Concentration of analytes in matrix

$\bar{C}p$ = Initial concentration of analytes

$\bar{C}o$ = Final concentration of analytes after testing

Limit of detection (LOD): This is response output or value above which it can be confirmed to be true with a stated level of confidence (ISO 6107-2, 2006.)

Limit of quantification (LOQ) is achieved from an appropriate standard sample which may be gotten from the last calibration point on the calibration curve without the blank sample (ISO 6107-2, 2006.)

Standard deviation is used to measure on how values are distributed about a mean of set of numbers. Standard deviation (s) for a sample is given by:

$$S = \sqrt{\frac{\Sigma(\mathcal{X} - \bar{\mathcal{X}})}{N-1}}$$

LOD = $3S$

LOQ = $6S$

Precision: This is closeness of agreement between measurement results gotten under condition of accord. Thus, precision depends on the distribution of prearranged order of errors and does not relate to absolute value. Results are obtained from more than one day of analysis and it is calculated by the following formula:

$$V = \frac{S}{\bar{X}} \times 100\%$$

S = Samples collected from more than one day

$\bar{X}$= Mean value

Trueness: This is closeness of agreement between expected test results and the absolute value. In general, the recognized reference value is replaced for true value (ISO 6107-2, 2006.). Hence, trueness can be calculated by the formula:

$$P = \bar{X} - X_R$$

$$P\% = \frac{\bar{X}}{X_R} - X_R \times 100\%$$

X_R = Expected value (0.1)

$\bar{X}$ = Mean value

9 EXPERIMENTAL SECTION FOR DETERMING OF PHENOLS

This chapter gives an overview of all analytical equipments, materials, chemicals used in this project. The experiments were performed in Water and Soil Testing Laboratory in Poznan, Poland. This method is suitable for determination of phenol, 4-nitrophenol, 2, 4-dinitrophenol, 2-chlorophenol, 2-nitrophenol, 2,4-dimethylphenol, 4-chloro-3-methylphenol, 2-methyl-4,6-dinitrophenol, 2,4,6-trichlorophenol, 2,4-dichlorophenol and pentachlorophenol in tap water using high performance liquid chromatography (HPLC) with UV-detector after solid phase extraction.

Principle: Phenols which are present in water are extracted to an appropriate stationary phase. The elution process of analytes is done using some organic solvent and the purity of the solvent is suitable for HPLC analysis. Phenols are separated on HPLC column using gradient, identification of analytes and quantification is done with UV-detector with optimization of maximum absorption of each analytes.

Interferences: All compound present in water samples having the same maximum absorption and the same retention time have the possibility of interfering with the analytes.

Reagent: All chemicals of analytical purity should be used to prevent additional contamination. Thus, the glass purity and the purity of chemicals need to be checked by analyzing blank sample.

Compounds used for extraction and HPLC analysis: Acetonitrile (CH_3CN), distilled water, methanol (CH_3OH), sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) to inactive the free chloride present in water sample, nitric acid (suprapur: a stage of purity). Standard stock solution with a concentration of 2000 mg/l of each phenol must be kept in dark at low temperature and should be protected from evaporation, also to avoid contact with skin.

TABLE 6. Chemical and solvent details

Name	**Producer**	**Details**
604 phenols calibration mix	Restek	2000 µg/ml each in methanol
Acetonitrile	Merck KGaA	2.5 l
Methanol	Merck KGaA	2.5 l
Distilled water	Millipore GmbH	Milli-Q Plus
Acetic acid	Merck KGaA	2.5 l (100%-vol)
Nitirc acid	Merck KGaA	1000 ml (100%-vol)
Tap water	Aquanet	

Apparatus: The usual laboratory apparatus and in particular; SPE columns filled with appropriate stationary phase.

TABLE 7. Description of all analytical equipments

Name	**Producer**	**Details**
SPE vacuum unit	Mallinckrodt Baker	SPE-12 G vacuum with tap
SPE column	J. T. Baker	
Glass vial		5 ml, 50 ml
Microlitre syringes (Chromatography syringes)	Hamilton thermoscientific	50 µl
Glass material	Schott duran	500 ml light brown sample bottles
Graduated pipette	Eppendorf	100-1000 µl
Measuring cylinder	Simax Czech republic	500 ml
Beakers	Simax Czech republic	50-250 ml
Transferpipette	Eppendorf	0.5-50 µl
Filter paper		Nylon 0.45 µm

Standard for calibration curve: Standard for calibrations were prepared by using standard stock solution to achieve 5 calibration solutions distributed as evenly as possible over an expected working range. However, checking the accuracy of calibration curve, some standard solution from manufacturer is used independently and the curves were checked at 2 different levels.

HPLC systems containing UV-detector with photodiode array detector (DAD) and appropriate software for data collection. In general, it consists of the following: eluent reservoir, a degassing unit, HPLC pump with gradient system, sample injection system, separation column, photodiode array detector (DAD) capable for UV-VIS measurement from 200 nm to 800 nm.

Quality requirement for the separation column: A typical HPLC column with length up to 300 mm, internal diameter from 2 mm to 4.6 mm packed with reverse phase and a size of particles from 3 µm to 5 µm.

TABLE 8. HPLC operating conditions (Confidential)

HPLC condition: The HPLC system was set up according to the manufacturer's instruction. Eluent was run for few minutes to ensure that the baseline is stable.

Calibration checking: Standard solutions of different phenols were measured to lower and upper working range making sure the calibration curve remain valid before blank and other samples are measured.

Detection, confirmation and identification: Peaks for particular phenols are identified by comparing the retention time with those from calibration solutions. Thus, changes of retention time should not exceed ±10% within a batch. The occurrence of peaks in chromatogram shows the probability of presence of analytes. To ensure a peak comes from analytes, UV spectrum of the analyte is compared with UV spectrum of calibration standard. (ISO/IEC.nr 35,1989.)

Quantification: The concentration of analytes was calculated taken into account dilution steps and recovery which was calculated during validation process. The concentration can be calculated by the following formula using software:

$$C_i = \frac{((y_i - b_i) \times V_e)}{a_i \times V_i \times \eta_i}$$

C_i= Concentration of analytes in mg/l

y_i= Peak area

b_i= Offset

a_i= Slope of the curve

η_i= Recovery of analytes

$\mathcal{V}_e$= Volume eluate

$\mathcal{V}_s$= Volume of sample

Table 9 gives a concise view on the performance characteristics of the method calculated during validation process showing the working range of each parameter. The recovery of each parameter should be within the range of 80-110% and the working range of each phenol between the ranges of 0.003-0.250 mg/l to meet the requirement according to standard (Polish act which describes the highest acceptable level of 2,4,6 trichlorophenol in water) .

TABLE 9. Performance characteristic of the method calculated during validation process

Parameter	Results (unit)	
Working range	phenol	0.003-0.250 mg/l
	4-nitrophenol	0.002-0.250 mg/l
	2,4-dinitrophenol	0.0025-0.250 mg/l
	2-chlorophenol	0.003-0.250 mg/l
	2-nitrophenol	0.0025-0.250 mg/l
	2,4-dimethylphenol	0.0032-0.250 mg/l
	4-chloro-3-methylphenol	0.003-0.250 mg/l
	2-methyl-4,6-dinitrophenol	0.0012-0.250 mg/l
	2,4-dichlorophenol	0.0036-0.250 mg/l
	2,4,6-trichlorophenol	0.003-0.250 mg/l
	pentachlorophenol	0.003-0.250 mg/l
Linearity	phenol	r= 0.999
	4-nitrophenol	r= 0.999
	2,4-dinitrophenol	r= 0.999
	2-chlorophenol	r= 0.999
	2-nitrophenol	r= 0.999
	2,4-dimethylphenol	r= 0.999
	4-chloro-3-methylphenol	r= 0.999
	2-methyl-4,6-dinitrophenol	r= 0.999
	2,4-dichlorophenol	r= 0.999
	2,4,6-trichlorophenol	r=0.999
	pentachlorophenol	r=0.999
Limit of detection	For sample with low content	$G_w = 3s$
	phenol	G_w= 0.0007
	4-nitrophenol	G_w= 0.0005
	2,4-dinitrophenol	G_w= 0.0006
	2-chlorophenol	G_w= 0.00075
	2-nitrophenol	G_w= 0.0006
	2,4-dimethylphenol	G_w= 0.0008
	4-chloro-3-methylphenol	G_w= 0.0007
	2-methyl-4,6-dinitrophenol	G_w =0.003
	2,4-dichlorophenol	G_w= 0.0009
	2,4,6-trichlorophenol	G_w= 0.0007
	pentachlorophenol	G_w= 0.0007
Precision	phenol	v = 3.81%
	4-nitrophenol	v= 3.73%
	2,4-dinitrophenol	v= 3.48%
	2-chlorophenol	v= 3.81%
	2-nitrophenol	v= 3.24%
	2,4-dimethylphenol	v= 7.17%
	4-chloro-3-methylphenol	v= 3.87%
	2-methyl-4,6-dinitrophenol	v=3.17%
	2,4-dichlorophenol	v= 4.19%
	2,4,6-trichlorophenol	v=3.49%
	pentachlorophenol	v=3.41%

TABLE 9. CONTINUE

Parameter	**Result (unit)**	
Correctness/ruggedness	phenol	p= -0.48%
	4-nitrophenol	p= -1.86%
	2,4-dinitrophenol	p=-2.06%
	2-chlorophenol	p=0.23%
	2-nitrophenol	p=-2.08%
	2,4-dimethylphenol	p=4.98%
	4-chloro-3-methylphenol	p=-0.53%
	2-methyl-4,6-dinitrophenol	p=-1.99%
	2,4-dichlorophenol	p=2.48%
	2,4,6-trichlorophenol	p=-2.24%
	pentachlorophenol	p=-1.24%
Recovery	For the entire operating range in water	
	phenol	f= 95.7%
	4-nitrophenol	f=99.1%
	2,4-dinitrophenol	f=99.2%
	2-chlorophenol	f= 96.0%
	2-nitrophenol	f= 99.0%
	2,4-dimethylphenol	f= 78.1%
	4-chloro-3-methylphenol	f= 96.3%
	2-methyl-4,6-dinitrophenol	f= 98.6%
	2,4-dichlorophenol	f=91.5%
	2,4,6-trichlorophenol	f= 98.7%
	pentachlorophenol	f = 90.9%

9.1 SOLID PHASE EXRACTION OF PHENOLS FROM MATRIX

This method is suitable for tap water only but can be tested for fitness for purpose regarding waste water, river water and lake water.

Sample pretreatment: A clean dark brown bottle for sampling with volume 500 ml to 1000 ml was filled with water to the brim with no free space to avoid evaporation of analytes. Thus, 50 mg of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) was added to the bottle to avoid reaction of analytes and some free chlorine that might be present in the water and 1 ml nitric acid (HNO_3) to the sample for preservation with pH < 7. Thus all samples were cooled, kept in dark and the extraction was done within 48hours.

TABLE 10. Sample preparation procedure

FIELD	Environmental
SAMPLE	604 Phenols calibration mix; 2000 µl/ml each in methanol
MATRIX	Tap water
EXTRACTION COLUMN	(Confidential)
SAMPLE PREPARATION	25 µl of reference material was added to 500 ml of water sample when checking recovery. 1 ml of HNO_3 was added to the sample for preservation
COLUMN CONDITIONING	(Confidential)
SAMPLE ADDITION	With vacuum off; 500 ml sample was connected to the column through a rubber tube. Attach SPE-12 G reservoir to the column, turn on the valve to allow extraction to occur at a steady flow rate
SAMPLE ELUTION	For HPLC analysis; vacuum dry the column between 5-10 minutes.
ANALYTICAL METHOD	HPLC with UV detector for analytes analysis

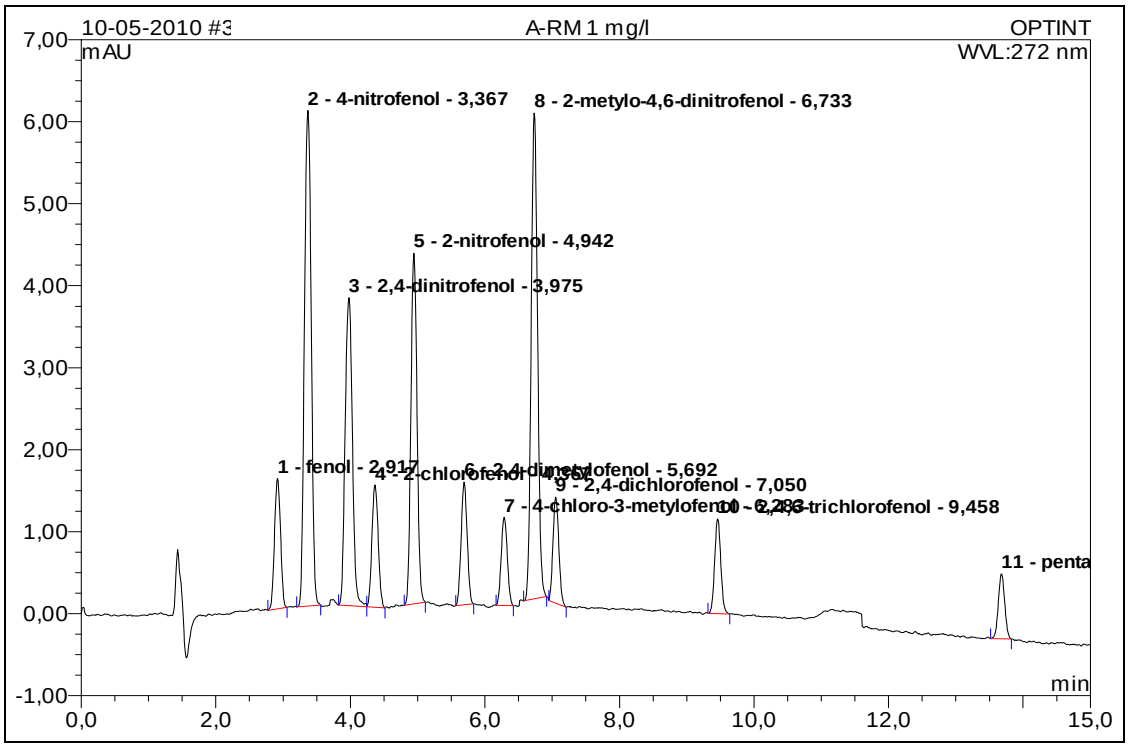
10 RESULTS AND DISCUSSIONS

This research is base on SPE process and the method was validated on a particular column to test water samples on 11 compounds of phenols using phenols calibration mix. However, the aim is to determine if there are other available columns that are suitable for the method as well as different matrixes. Before any analysis, the accuracy of calibration curves was checked to ensure they are still valid with a standard solution of 1 mg/l and 20 mg/l. The results from the analysis of phenols from standard solutions to test calibration curves using this validated method can be seen in Table 10.

TABLE 11. Results of the 11 phenols obtained from standard solution of 1 mg/l

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount mg/l	Type
1	2.92	Phenol	1.588	0.166	5.19 0	1.034	BMB
2	3.37	4-nitrophenol	6.042	0.702	21.95	1.025	BMB
3	3.98	2,4-dinitrophenol	3.755	0.469	14.68	1.031	BM
4	4.37	2-chlorophenol	1.491	0.161	5.050	1.035	MB
5	4.94	2-nitrophenol	4.276	0.445	13.93	1.033	BMB
6	5.69	2,4-dimethylphenol	1.495	0.155	4.850	1.004	BMB
7	6.28	4-chloro-3-methylphenol	1.075	0.112	3.520	1.014	BMB
8	6.73	2-methyl-4,6-dinitrophenol	5.925	0.647	20.25	1.020	BMB
9	7.05	2,4-dichlorophenol	1.292	0.131	4.080	0.972	BMB
10	9.46	2,4,6-trichlorophenol	1.154	0.121	3.790	1.017	BMB
11	13.68	Pentachlorophenol	0791	0.087	2.710	1.002	BMB
Total:			28.884	3.197	100.00	11.186	

Table 11 presents the results of the 11 phenols obtained from standard solution of 1 mg/l. The calculation of concentration of the compounds are based on the area of peaks (mAU*min). The nominal values of concentrations of each phenol were 1 mg/l to check the accuracy of calibration curve at the lower working range, thus the result gotten from the standard solution shows an excellent accuracy of all calibration curves of all phenols on the lower level.



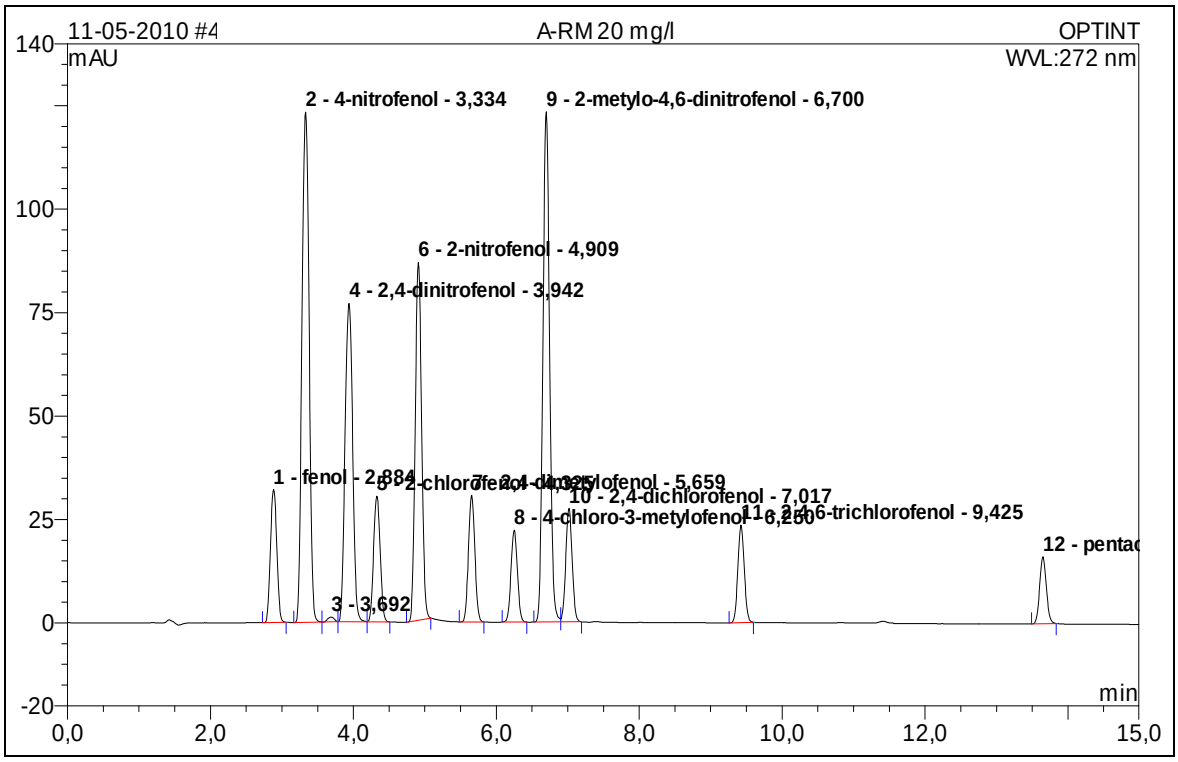
GRAPH 8. Typical chromatogram obtained from a standard solution of 1 mg/l

The chromatogram in Graph 8 refers to that obtained from a standard solution. The peak at the beginning of the chromatogram comes from an unknown contamination from matrix which does not affect the separation of analytes. Thus, the baseline is not so smooth due to lower concentration of standard solution.

TABLE 12. Results of the 11 phenols obtained from standard solution of 20 mg/l

No.	Ret.Time Min	Peak Name	Height mAU	Area mAU*mn	Rel.Area %	Amount mg/l	Type
1	2.88	Phenol	32.115	3.328	5.11	20.743	BMB
2	3.33	4-nitrophenol	123.287	14.224	21.83	20.777	BM
3	3.69	n.a.	1.128	0.114	0.18	–	Mb
4	3.94	2,4-dinitrophenol	76.893	9.487	14.56	20.854	bM
5	4.33	2-chlorophenol	30.335	3.238	4.97	20.756	MB
6	4.91	2-nitrophenol	86.467	8.914	13.68	20.677	BMB
7	5.66	2,4-dimethylphenol	30.600	3.194	4.90	20.690	BMB
8	6.25	4-chloro-3-methylphenol	22198	2.300	3.53	20.750	BMB
9	6.70	2-methyl-4,6-dinitrophenol	123.357	13.345	20.48	21.016	BM
10	7.02	2,4-dichlorophenol	27.404	2.829	4.34	21.055	MB
11	9.43	2,4,6-trichlorophenol	23.558	2.441	3.75	20.490	BMB
12	13.65	Pentachlorophenol	16.204	1.746	2.68	20159	BMB
Total:			593.544	65.160	100.00	227.967	

Table 12 shows the results of the 11 phenols obtained from standard solution of 20 mg/l. Hence, the calculation of concentrations of the compounds are based on the peak area (mAU*min). The nominal values of concentrations of each phenol were 20 mg/l to check the accuracy for calibration curves at the higher working range, thus the result gotten from standard solution shows an excellent accuracy of all calibration curves of all phenols on the higher level of working range.



GRAPH 9. Typical chromatogram obtained from a standard solution of 20 mg/l

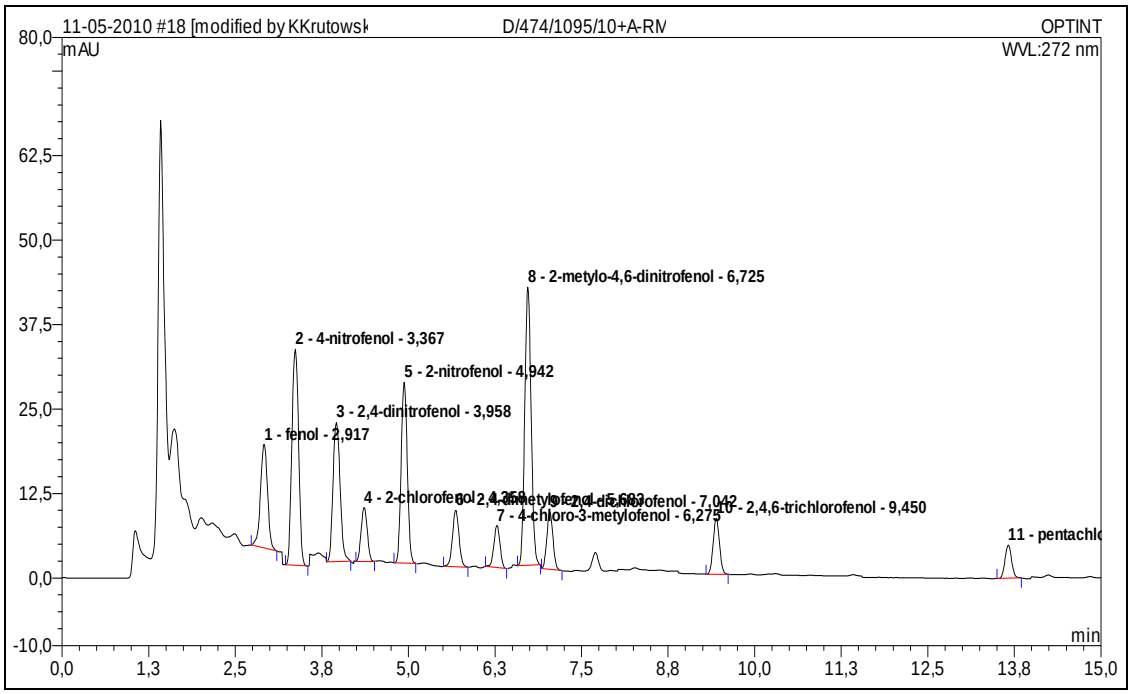
Graph 9 shows a typical chromatogram obtained from a standard sample containing the 11 phenols from water sample with a standard solution of 20 mg/l. The baseline is smooth due to high concentration of the standard solution.

Consequently, using the same validated method for the 11 phenols for water samples and the same solid phase extraction procedures, wastewater sample was also analyzed using the same columns to determine if the method is suitable for wastewater matrix. However, series of results was obtained after carrying out the analysis. The results from the analysis of phenols from spiked wastewater samples using this validated method can be seen in Table 13.

TABLE 13. Results obtained from spiked wastewater sample

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount mg/l	Type
1	2.92	Phenol	15.333	1.805	9.20	0.113	BMB
2	3.37	4-nitrophenol	31.962	3.592	18.32	0.052	BMB
3	3.96	2,4-dinitrophenol	20.586	2.446	12.47	0.054	BMB
4	4.36	2-chlorophenol	7.935	0.819	4.18	0.053	BMB
5	4.94	2-nitrophenol	26.765	2.740	13.97	0.064	BMB
6	5.68	2,4-dimethylphenol	8.369	0.909	4.64	0.059	BMB
7	6.28	4-chloro-3-methylphenol	6.197	0.630	3.21	0.057	BMB*
8	6.73	2-methyl-4,6-dinitrophenol	41.106	4.389	22.38	0.090	BMB*
9	7.04	2,4-dichlorophenol	8.463	0.868	4.42	0.065	BMB
10	9.45	2,4,6-trichlorophenol	8.255	0.873	4.45	0.093	BMB
11	13.66	Pentachlorophenol	4.914	0.539	2.75	0.062	BMB
Total:			179.885	19.611	100.00	0.761	

The Table 13 shows the results of the 11 phenols obtained from spiked wastewater samples with a particular column. Thus, the nominal value of concentration of each phenols were 0.1 mg/l but the results are less than 0.1 mg/l indicating that there are some serious interferences between matrix and analytes. Thus, recovery for this analysis needs to be calculated because recovery for this matrix is different. The chromatogram of wastewater shows that there are no phenols present within the working range.



GRAPH 10. Typical chromatogram from spiked wastewater sample

Graph 10 shows typical chromatogram from spiked wastewater sample. However, the baseline is quite different from that obtained from a standard samples due to low concentration of standard solution 0.1 mg/l used in the experiment. The peak at the beginning of the chromatogram comes from an unknown contamination from matrix which does not affect the separation of analytes.

However, for this research, four different columns were tested to determine their effect on analytes and which column has the ability to hold anaytes strongly; the ability of each column to retain analytes. Hence, the research was done using BAKERBOND spe^{TM} (Octadecyl en SDB); 6 ml disposable extraction columns packed with 500 mg octadecyl (C18 on top) en 200 mg styrene divinylbenzene copolymere (SDB), C18 polar plus 3000 mg/6 ml, QUART.AMINE 500 mg/ 3 ml and Octadecyl (C18) 500 mg/ 3 ml per column. Thus, after the analysis, it shows that two columns out of five have an excellent ability to retain analytes and the results obtained from the analysis shows that there are no phenols in the matrix (samples) due to strong bond of analytes on the stationary phase (column). Also, the results were not as excellent as the results obtained from the column used in the method which can be seen Table 21 in appendixes.

Nevertheless, the results obtained after analyzing different matrixes shows that phenols were not detected within the working range of the validated method. However, recovery testing performed using different matrixes (lake water) shows that the method is suitable for surface water analysis and the results can be seen in Table 22and 23 in appendixes.

11 CONCLUSIONS

This study is based on the SPE process, and the method employed was validated for tap water using phenol calibration mix which aims at checking whether there are other columns that could be used for the developed method as well as other matrixes. However, for the validated method five different columns were analyzed to determine their properties. The ability of each column to retain analytes and the results achieved show that C18, polar plus, Quart. Amine columns are not suitable for determination of phenols from water samples using HPLC with a UV-detector but C18/SDB columns can be use due to excellent recoveries of analytes which were between the working ranges of 80-110%.

Thus, the validated method was concluded to be suitable for analyzing different matrixes which gave excellent recoveries within the working range. It can also be concluded that phenols are not present in lake water but the validated method is suitable for analyzing different water matrixes. Samples were collected from Uli Lake (52° 25' 38''; 17° 22' 04''), Baba Lake (52° 25' 14''; 17° 21' 57''), Dobre Lake (52° 28' 19''; 17° 17' 41''), Debiniec Lake (52° 27' 59''; 17° 13' 46'') and Malta Lake. Thus, every sample was analyzed twice for accuracy.

As regards waste water, the recoveries were very low due to interferences between matrix and analytes. Nevertheless, the results from samples differ greatly making it impossible to calculate the mean value of recoveries. A different approach is needed for determining concentrations. Thus, one approach might be to calculate recovery for each waste water sample separately.

The experimental results show that phenols are not present in water within the working range of concentrations 0.003-0.25 mg/l. The guideline base on drinking water directive for Polish regulation act shows that the concentration of 2, 4, 6-trichlorophenol should be less than 0.2 mg/l. In every tested tap water, phenols were not detected showing that water meets the requirement of Polish act of drinking water quality. In comparison with the result from the experiments obtained from spiked water samples and that of standard samples, it show that the concentration was 0.093 mg/l for 2,4,6-trichlorophenol which supports the conclusion that the method is suitable for the determination of 2,4,6-trichlorophenol and the rest of phenols in water using HPLC with a UV-detector.

Referring to the research questions of this study, the developed method is trustworthy and suitable for the determination of phenols in water. The method was checked for accuracy using internal quality control (checking the calibration curves and recovery testing). However, a known volume (25 µl) reference material of concentration 2000 mg/l phenol mix was diluted in order to achieve an end concentration of 0.1 mg/l of each phenol. The reference material was used to create spiked samples to check recoveries of each phenol as well as to determine if the error present was less or greater than 10% after analysis, for the method to be valid. Also, using an external quality control for checking the results of phenols obtain from inter-laboratory proficiency testing (LGC standard).

In suggestion for further research concerning method validation, more research should be conducted on method for determination of drugs and hormones present in water due to large consumption of these chemical compounds by humans.

This research has ignited in me a further interest and motivation in method development and validation not only in the environmental sector but also in forensic sciences. It has vastly enhanced my knowledge in the field of environmental analysis, research study which will enable me to overcome future challenges in working life.

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Appendix 1/1

TABLE 14. Results from water samples after experiment using validated method in mg/l

No.	Name	P	4-NP	2,4-DNP	2-CP	2-NP	2,4-DMP	4-C-3-MP	2-M-4,6-DNP	2,4-DCP	2,4,6-TCP	PCP	Time	Dil.Fac
1	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	12:09	1.0
2	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	12:31	1.0
3	A-RM 1 mg/l	1.0335	1.0248	1.0312	1.0352	1.0326	1.0045	1.0142	1.0195	0.9716	1.0173	1.0017	12:53	1.0
4	A-RM 20 mg/l	20.7535	20.8597	20.8984	20.7701	20.6894	20.6593	20.7717	21.0424	21.0340	20.5245	20.2509	13:14	1.0
5	"0"	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13:36	1.0
6	woda wodoc.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13:58	1.0
7	woda wodoc + A-RM	0.1035	0.1010	0.0995	0.1039	0.1003	0.1180	0.1030	0.1002	0.1052	0.0990	0.0983	14:20	1.0
8	H2O/10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	14:42	1.0
9	H2O+A-RM/10	0.1048	0.1006	0.1000	0.1022	0.0978	0.1182	0.1029	0.1001	0.1080	0.0978	0.0986	15:04	1.0
10	H2O+A-RM/10	0.0973	0.0946	0.0959	0.0953	0.0941	0.1158	0.0953	0.0961	0.0961	0.0935	0.0973	15:26	1.0
11	D/477/1094/10	0.3114	n.a.	0.0636	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15:48	1.0
12	D/477/1095/10	0.1435	n.a.	0.0445	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:10	1.0
13	D/477/1095/10"	0.1449	n.a.	00397	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:32	1.0
14	STOP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:50	1.0

Definition of abbreviations

P : Phenol	4-C,3-MP: 4-chloro-3-methylphenol
4 –NP : 4- nitrophenol	2-M-4,6-DNP: 2-methyl-4,6-dinitrophenol
2,4-DNP : 2,4- dinitrophenol	2,4-DCP: 2,4-dichlorophenol
2-CP : 2-chlorophenol	2,4,6-TCP: 2,4,6-trichlorophenol
2-NP: 2-nitrophenol	PCP: pentachlorophenol
2,4-DMP: 2,4- dimethylphenol	Dil. Fac: Dilution factor

Appendix 1/2

TABLE 15. Results from water samples during second experiment in mg/l

No.	Name	P	4-NP	2,4-DNP	2-CP	2-NP	2,4-DMP	4-C-3-MP	2-M-4,6-DNP	2,4-DCP	2,4,6-TCP	PCP	Time	Dil.Fac.
1	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15:53	1.0
2	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:14	1.0
3	A-RM 1 mg/l	1.0287	1.0266	1.0132	1.0417	1.0312	1.0145	1.0287	1.0500	1.0123	1.0320	0.9581	16:36	1.0
4	A-RM 20 mg/l	20.7429	20.7773	20.8541	20.7562	20.6775	20.6902	20.7496	21.0155	21.0554	20.4899	20.1587	16:58	1.0
5	H2O	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	17:20	1.0
6	H2O+A-RM	0.0929	0.0984	0.1029	0.0992	0.1021	0.0971	0.0993	0.1085	0.1016	0.1033	0.1019	17:42	1.0
7	H2O+A-RM	0.0926	0.0983	0.1015	0.0984	0.1021	0.0971	0.0993	0.1084	0.1018	0.1035	0.1018	18:04	1.0
8	H2O+A-RM	0.0894	0.0944	0.0967	0.0928	0.0962	0.0891	0.0900	0.1013	0.0874	0.0935	0.0949	18:26	1.0
9	H2O+A-RM	0.0883	0.0931	0.0947	0.0910	0.0949	0.0878	0.0894	0.1026	0.0868	0.0921	0.0936	18:48	1.0
10	H2O+A-RM	0.0713	0.0775	0.0790	0.0774	0.0831	0.0723	0.0738	0.0906	0.0743	0.0845	0.0947	19:10	1.0
11	H2O+A-RM	0.0710	0.0776	0.0790	0.0774	0.0831	0.0721	0.0738	0.0930	0.0741	0.0845	0.0953	19:32	1.0
12	H2O+A-RM	0.1022	0.1011	0.1021	0.0998	0.0968	0.1020	0.1020	0.0940	0.0974	0.0960	0.0840	19:54	1.0
13	H2O+A-RM	0.1020	0.1011	0.1021	0.1002	0.0968	0.1019	0.1022	0.0960	0.1011	0.0961	0.0843	20:16	1.0
14	D/474/1094/10 +A-RM	0.2138	0.0616	0.0775	0.0640	0.0733	0.0697	0.0661	0.0768	0.0715	0.0802	0.0595	20:37	1.0
15	D/474/1094/10 +A-RM	0.2157	0.0616	0.0774	0.0637	0.0736	0.0699	0.0660	0.0770	0.0730	0.0800	0.0593	20:59	1.0
16	D/474/1094/10 +A-RM	0.2142	0.0647	0.0834	0.0635	0.0739	0.0725	0.0672	0.0801	0.0720	0.0813	0.0661	21:21	1.0
17	D/474/1094/10 +A-RM	0.2131	0.0649	0.0836	0.0639	0.0739	0.0729	0.0671	0.0802	0.0702	0.0816	0.0664	21:43	1.0
18	D/474/1095/10 +A-RM	0.1125	0.0525	0.0538	0.0525	0.0636	0.0589	0.0568	0.0691	0.0646	0.0733	0.0623	22:05	1.0
19	D/474/1095/10 +A-RM	0.1129	0.0526	0.0541	0.0523	0.0636	0.0589	0.0574	0.0692	0.0641	0.0733	0.0614	22:27	1,0
20	D/474/1095/10 +A-RM	0.1532	0.0662	0.0690	0.0654	0.0753	0.0723	0.0679	0.0795	0.0722	0.0803	0.0657	22:49	1.0
21	D/474/1095/10 +A-RM	0.1526	0.0662	0.0684	0.0655	0.0753	0.0725	0.0679	0.0797	0.0729	0.0804	0.0655	23:11	1.0
22	STOP	n.a.	n.a.	0.3415	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23:29	1.0

From Table 14 and 15, it can be seen that phenols are not present in tap water from the working range of the method validation but might be present at a lower concentration other than the working range of 0.003-0.25 mg/l. However, further research was done using five different columns to test their retention ability of analytes. The results obtained from the different columns can be seen in Table 16, 17 and 18.

TABLE 16. Results obtained from a particular column on the first day of experiment in mg/l

No.	Name	P	4-NP	2,4-DNP	2-CP	2-NP	2,4-DMP	4-C-3-MP	2-M-4,6-DNP	2,4-DCP	2,4,6-TCP	PCP	Time	Dil.Fac.
1	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15:53	1.0
2	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:14	1.0
3	A-RM 1 mg/l	1.0236	1.0296	1.0156	1.0253	1.0261	1.0146	1.0108	1.0165	1.0095	1.0230	0.9813	16:36	1.0
4	A-RM 20 mg/l	20.7250	20.7252	20.7489	20.6811	20.6638	20.6408	20.7426	20.8128	20.7375	20.4492	20.1842	16:58	1.0
5	H2O	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	17:20	1.0
6	C18+SDB-1	0.0927	0.0982	0.1017	0.0984	0.1020	0.0964	0.0992	0.1400	0.1017	0.1317	0.1018	17:42	1.0
7	C18+SDB-1	0.0923	0.0981	0.1016	0.0983	0.1020	0.0963	0.0990	0.1398	0.1005	0.1311	0.1011	18:04	1.0
8	C18+SDB-1	0.0894	0.0940	0.0957	0.0924	0.0959	0.0886	0.0901	0.1328	0.0922	0.1182	0.0943	18:26	1.0
9	C18+SDB-1	0.0883	0.0927	0.0947	0.0910	0.0949	0.0873	0.0889	0.1311	0.0911	0.1168	0.0937	18:48	1.0
10	C18+SDB-1	0.0711	0.0771	0.0799	0.0785	0.0861	0.0734	0.0737	0.1189	0.0781	0.1068	0.0951	19:10	1.0
11	C18+SDB-1	0.0710	0.0773	0.0799	0.0782	0.0828	0.0717	0.0735	0.1190	0.0777	0.1069	0.0951	19:32	1.0
12	C18+SDB-1	0.1019	0.1008	0.1004	0.0992	0.0964	0.1012	0.1020	0.1235	1004	0.1218	0.0842	19:54	1.0
13	C18+SDB-1	0.1019	0.1008	0.1006	0.0994	0.0965	0.1013	0.1015	0.1237	0.1000	0.1218	0.0840	20:16	1.0
14	D/474/1094/10 +A-RM	0.2204	0.0596	0.0757	0.0647	0.0739	0.0711	0.0634	0.1005	0.0853	0.1003	0.0601	20:37	1.0
15	D/474/1094/10 +A-RM	0.2221	0.0596	0.0755	0.0662	0.0745	0.0723	0.0633	0.1007	0.0853	0.0996	0.0600	20:59	1.0
16	D/474/1094/10 +A-RM	0.2202	0.0627	0.0816	0.0666	0.0746	0.0750	0.0647	0.1048	0.0855	0.1017	0.0671	21:21	1.0
17	D/474/1094/10 +A-RM	0.2193	0.0625	0.0821	0.0668	0.0749	0.0749	0.0647	0.1050	0.0860	0.1010	0.0670	21:43	1.0
18	D/474/1095/10 +A-RM	0.1156	0.0523	0.0588	0.0527	0.0638	0.0673	0.0561	0.0902	0.0733	0.0912	0.0603	22:05	1.0
19	D/474/1095/10 +A-RM	0.1154	0.0523	0.0590	0.0527	0.0640	0.0673	0.0553	0.0904	0.0732	0.0915	0.0610	22:27	1.0
20	D/474/1095/10 +A-RM	0.1574	0.0654	0.0750	0.0658	0.0755	0.0802	0.0653	0.1040	0.0842	0.0994	0.0644	22:49	1.0
21	D/474/1095/10 +A-RM	0.1569	0.0652	0.0749	0.0658	0.0756	0.0808	0.0660	0.1040	0.0842	0.0998	0.0641	23:11	1.0
22	STOP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23:29	1.0

TABLE 17. Results obtained from different columns from the second day of experiment in mg/l

No.	Name	P	4-NP	2,4-DNP	2-CP	2-NP	2,4-DMP	4-C-3-MP	2-M-4,6-DNP	2,4-DCP	2,4,6-TCP	PCP	Time	Dil.Fac.
1.	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15:26	1.0
2.	A-RM 1 mg/l	1.0603	1.0542	1.0523	1.0641	1.0522	1.0588	1.0363	1.0401	0.9797	1.0367	1.0605	15:48	1.0
3.	A-RM 20 mg/l	20.7483	20.7435	20.7504	207087	20.7200	20.6308	20.7115	20.7904	20.5789	20.4534	20.1890	16:10	1.0
4.	H2O	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:32	1.0
5.	C18	0.0097	0.0307	0.0054	0.0428	0.0590	0.1456	0.1826	0.0249	0.1758	0.1824	0.1776	16:54	1.0
6.	C18	0.0056	0.0197	0.0362	0.0256	0.0354	0.0806	0.0946	0.0966	0.0938	0.0953	0.0915	17:16	1.0
7.	C18	0.0048	0.0178	0.0136	0.0223	0.0312	0.0730	0.0897	0.0632	0.0812	0.0902	0.0868	17:38	1.0
8.	C18	0.0047	0.0153	0.0054	0.0214	0.0289	0.0727	0.0923	0.0146	0.0873	0.0926	0.0916	17:59	1.0
9.	C18	0.0048	0.0154	0.0054	0.0215	0.0289	0.0731	0.0929	0.0148	0.0896	0.0938	0.0919	18:21	1.0
10.	C18	0.0057	0.0197	0.0365	0.0257	0.0356	0.0811	0.0958	0.0972	0.0957	0.0959	0.0923	18:43	1.0
11.	C18	0.0052	0.0162	0.0030	0.0229	0.0309	0.0780	0.0970	0.0131	0.0916	0.0967	0.0952	19:05	1.0
12.	C18	0.0047	0.0178	0.0136	0.0225	0.0314	0.0729	0.0895	0.0631	0.0810	0.0897	0.0866	19:27	1.0
13.	POLARPLUS	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	19:49	1.0
14.	POLARPLUS	0.0407	0.0910	0.0514	0.0896	0.0898	0.0906	0.0913	0.0922	0.0911	0.0906	0.0812	20:11	1.0
15.	POLARPLUS	0.0335	0.0921	0.0924	0.0899	0.0900	0.0903	0.0911	0.0920	0.0918	0.0903	0.0658	20:33	1.0
16.	POLARPLUS	0.0404	0.0908	0.0512	0.0897	0.0900	0.0906	0.0914	0.0917	0.0912	0.0909	0.0798	20:55	1.0
17.	POLARPLUS	0.0324	0.0912	0.0904	0.0869	0.0871	0.0874	0.0890	0.0901	0.0890	0.0880	0.0692	21:17	1.0
18.	POLARPLUS	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	21:39	1.0
19.	POLARPLUS	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	22:00	1.0
20.	POLAR PLUS	0.0435	0.0893	0.0459	0.0873	0.0878	0.0885	0.0894	0.0899	0.0895	0.0887	0.0782	22:22	1.0
21.	POLAR PLUS	0.0433	0.0889	0.0455	0.0864	0.0871	0.0874	0.0880	0.0891	0.0883	0.0879	0.0797	22:44	1.0
22.	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23:06	1.0
23.	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	00:12	1.0
24.	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	00:56	1.0
25.	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0,0004	n.a.	n.a.	n.a.	01:18	1.0
26.	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0,0005	n.a.	n.a.	0,0093	01:40	1.0
27.	QUART.AMINE	n.a.	0.0022	0.0008	n.a.	n.a.	n.a.	n.a.	0.0005	n.a.	n.a.	0.0106	02:02	1.0
28.	STOP	n.a.	n.a.	n.a.	n.a.	18.9375	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	02:20	1.0

TABLE 18. Results obtained from different columns from the third day of experiment in mg/l

No.	Name	P	4-NP	2,4-DNP	2-CP	2-NP	2,4-DMP	4-C-3-MP	2-M-4,6-DNP	2,4-DCP	2,4,6-TCP	PCP	Time	Dil.Fac.
1	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	14:50	1.0
2	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15:12	1.0
3	A-RM 1 mg/l	1.0447	1.0528	1.0527	1.0802	1.0545	1.0384	1.0465	1.0847	10305	1.0455	1.0126	15:33	1.0
4	A-RM 20 mg/l	20.7162	20.7168	20.8459	20.7476	20.6485	20.6803	20.7563	21.0099	21.,0494	20.5959	20.4147	15:55	1.0
5	"0"	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:17	1.0
6	C18	0.0028	0.0087	0.0014	0.0131	0.0210	0.0333	0.0365	0.0114	0.0358	0.0385	0.0463	16:39	1.0
7	C18	0.0076	0.0278	0.0054	0.0373	0.0502	0.0951	0.1064	0.0308	0.1040	0.1044	0.1010	17:01	1.0
8	C18	0.0080	0.0279	0.0053	0.0377	0.0472	0.0933	0.1022	0.0225	0.1014	0.0977	0.0905	17:23	1.0
9	C18	0.0052	0.0166	0.0027	0.0255	0.0367	0.0646	0.0710	0.0155	0.0714	0.0764	0.0836	17:45	1.0
10	SDB-2	0.0955	0.0943	0.0955	0.0917	0.0900	0.0914	0.0921	0.0899	0.0873	0.0879	0.0775	18:07	1.0
11	SDB-2	0.0625	0.0671	0.0739	0.0679	0.0779	0.0708	0.0742	0.0890	0.0748	0.0848	0.0873	18:29	1.0
12	SDB-2	0.0768	0.0840	0.0858	0.0825	0.0879	0.0841	0.0884	0.0949	0.0911	0.0949	0.0931	18:51	1.0
13	SDB-2	0.0947	0.0965	0.0960	0.0938	0.0905	0.0930	0.0938	0.0895	0.0884	0.0903	0.0802	19:13	1.0
14	POLARPLUS	0.0213	0.0585	0.0178	0.0614	0.0672	0.0680	0.0687	0.0685	0.0706	0.0790	0.0896	19:34	1.0
15	POLARPLUS	0.0293	0.0832	0.0262	0.0917	0.1042	0.1060	0.1083	0.1081	0.1123	0.1183	0.0785	19:56	1.0
16	POLARPLUS	0.0394	0.0885	0.0301	0.0907	0.0938	0.0920	0.0918	0.0947	0.0939	0.0948	0.0791	20:18	1.0
17	POLARPLUS	0.0362	0.0801	0.0263	0.0801	0.0820	0.0823	0.0828	0.0804	0.0803	0.0870	0.0773	20:40	1.0
18	C18/SDB	0.0841	0.0890	0.0892	0.0865	0.0866	0.0867	0.0880	0.0909	0.0896	0.0888	0.0864	21:02	1.0
19	C18/SDB	0.0711	0.0780	0.0829	0.0785	0.0824	0.0729	0.0742	0.0907	0.0736	0.0829	0.0892	21:24	1.0
20	C18/SDB	0.0545	0.0580	0.0664	0.0582	0.0660	0.0541	0.0568	0.0676	0.0567	0.0673	0.0788	21:46	1.0
21	C18/SDB	0.0784	0.0830	0.0858	0.0795	0.0804	0.0786	0.0804	0.0822	0.0773	0.0817	0.0812	22:08	1.0
22	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	0.0005	n.a.	n.a.	0.0006	n.a.	0.0027	0.0101	22:30	1.0
23	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.0005	n.a.	n.a.	n.a.	22:52	1.0
24	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.0004	n.a.	n.a.	n.a.	23:14	1.0
25	QUART.AMINE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23:35	1.0
26	STOP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23:53	1.0

The results from three different days of experiments were collected to calculate recoveries (%F) of 11 phenols so as to compare which column gives better recovery of analytes and which one is not suitable for analyzing phenols in water from the validated method.

TABLE 19. Example of the recoveries calculated for evaluation of particular compounds

	C_p	C_o	C_z
1	0.0000	0.100	0.0896
2	0.0000	0.100	0.0785
3	0.0000	0.100	0.0773
4	0.0000	0.100	n. a
5	0.0000	0.100	0.0812
6	0.0000	0.100	0.0658
7	0.0000	0.100	0.0798
8	0.0000	0.100	0.0798
9	0.0000	0.100	0.0692
10	0.0000	0.100	n. a
11	0.0000	0.100	n. a
12	0.0000	0.100	0.0782
Averages	0.0000	0.100	0.0787

$$\%F = \frac{\overline{C_z} - \overline{C_p}}{\overline{C_o}} \times 100\%$$

$\overline{C}z$ = Concentration of analytes in matrix from more than one day in mg/l

$\overline{C}p$ = Initial concentration of analytes in mg/l

Co = Final concentration of analytes after testing in mg/l

Appendix 1/3

TABLE 20. Recoveries from 11 phenols obtained from different columns in percentage (%F)

Compound	C18	Polar plus	C18/SDB	Quart. Amine
Phenol	5.6	43.3	85.638	n. a
4-nitrophenol	20.5	80.1	89.9095	n. a
2,4-dinitrophenol	12.1	45.5	92.0432	n. a
2-chlorophenol	27.7	80.0805	89.0856	n. a
2-nitrophenol	36.4	87.0584	91.4638	n. a
2,4-dimethylphenol	86.5	82.3429	86.77	n. a
4-chloro-3-methylphenol	104.3	87.9785	88.2274	n. a
2-methyl-4,6-dinitrophenol	39.0	80.359	117.5027	n. a
2,4-dichlorophenol	100.1	88.316	89.2791	n. a
2,4,6-trichlorophenol	103.9	86.9554	109.8538	n. a
Pentachlorophenol	99.0	79.7302	90.4161	n. a

From the experimental research, it can be seen that the Quart. Amine columns did not yield any results which were not suitable for the validated method because analytes were strongly bonded to it which made it impossible to elute and detect phenols in matrix. However, C18/SDB give an excellent recoveries of phenols which were in the range between 80-110% expect from 2-methyl-4, 6-dinitrophenol compound which exceed the range and has 117.5% recovery probably due to interferences of the sample or the column. Thus C18 and Polar plus columns did not give excellent recoveries because not all compounds met the recovery range. Furthermore, for this validated method, C18, polar plus, Quart. Amine columns are not suitable for determination of phenols from water sample using HPLC with UV-detector.

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TABLE 21. Parameters of calibration curve of standard solution of 1 mg/l

Sample name: A-RM 1 mg/L		Quantif. method: Phenol			Dilution factor: 1.000			
Vial number : BB2		Recording time: 11.5.2010 16.36			Sample factor: 1.000			
Sample type: Validate Control program: Phenol		Run time (min) : 14.99 Injection volume: 20.0			Sample amount:1.000 Wavelength & Bandwidth: n. a			
No.	Ret. Time min	Peak Name	Cal. Type	Points	Corr. Coeff. (%)	Offset	Slope	Curve
1	2.88	Phenol	Lin	7	99.9750	0.0000	0.1604	0.0000
2	3.33	4-nitrophenol	Lin	7	99.9772	0.0000	0.6846	0.0000
3	3.94	2,4-dinitrophenol	Lin	7	99.9760	0.0000	0.4549	0.0000
4	4.33	2-chlorophenol	Lin	7	99.9741	0.0000	0.1560	0.0000
5	4.91	2-nitrophenol	Lin	7	99.9763	0.0000	0.4311	0.0000
6	5.65	2,4-dimethylphenol	Lin	7	99.9773	0.0000	0.1544	0.0000
7	6.25	4-chloro-3-methylphenol	Lin	7	99.9786	0.0000	0.1108	0.0000
8	6.70	2-methyl-4,6-dinitrophenol	Lin	7	99.9752	0.0000	0.6350	0.0000
9	7.02	2,4-dichlorophenol	Lin	7	99.9483	0.0000	0.1344	0.0000
10	9.43	2,4,6-trichlorophenol	Lin	7	99.9776	0.0000	0.1191	0.0000
11	13.65	Pentachlorophenol	Lin	7	99.9786	0.0000	0.0866	0.0000
Average:					99.9740	0.0000	0.2843	0.0000

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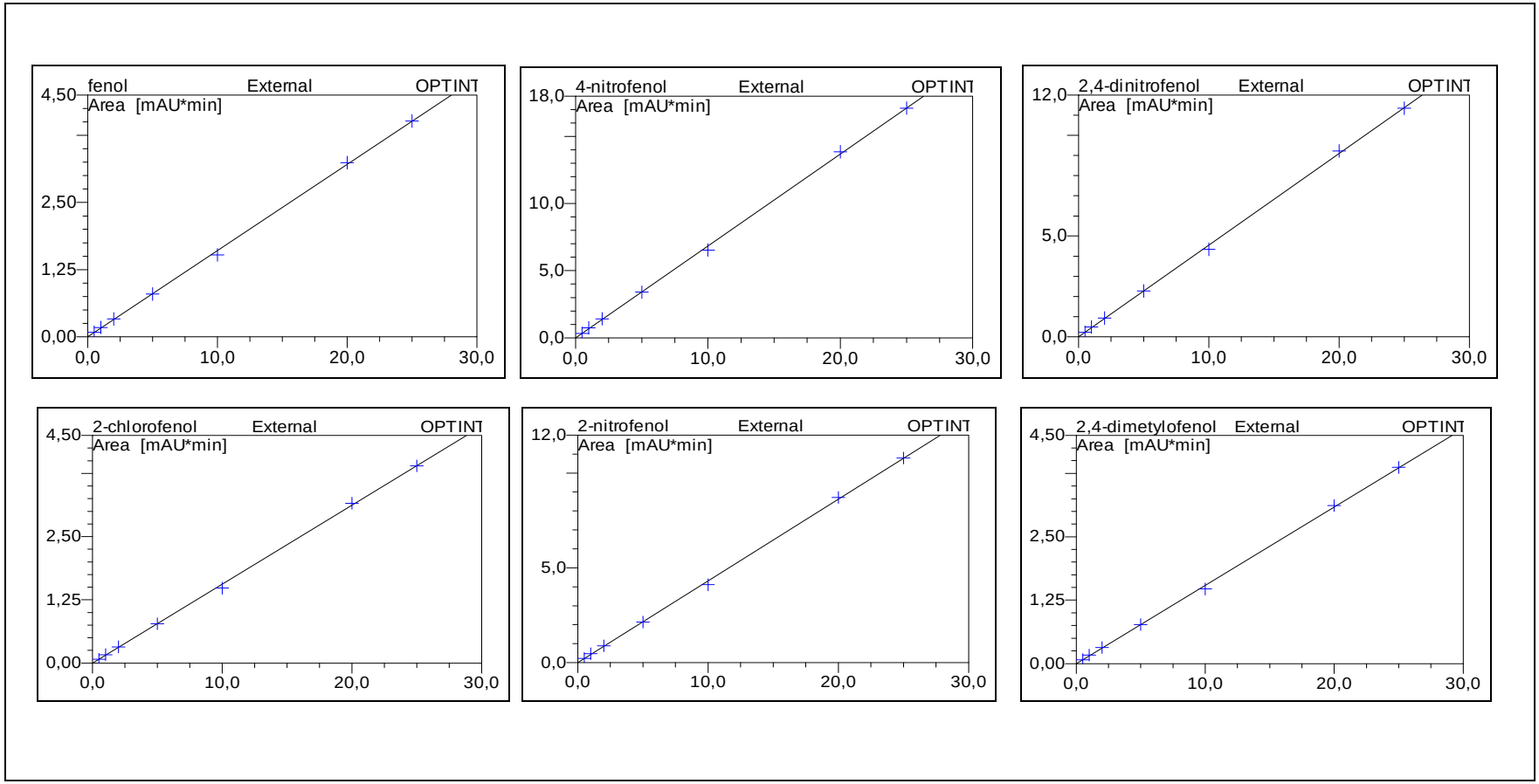
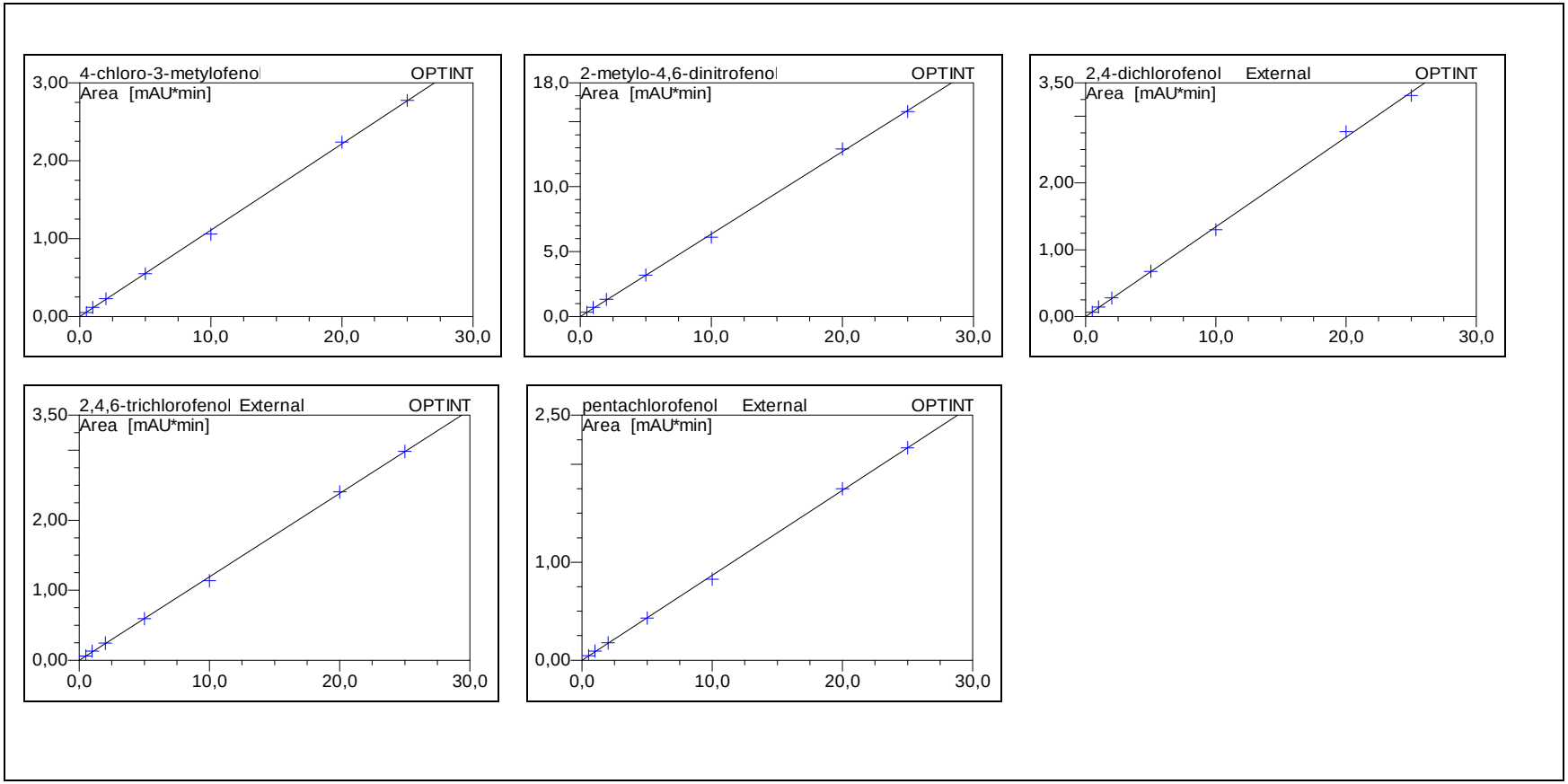


GRAPH 12. Calibration curves of 11 phenols from analysis

Appendix 1/5 continues



GRAPH 12. Calibration curves of 11 phenols from analysis

Appendix 1/6

TABLE 22.Results obtained from analysis of matrix from a particular lake

No.	Name	P	4-NP	2,4-DNP	2-CP	2-NP	2,4-DMP	4-C-3-MP	2-M-4,6-DNP	2,4-CP	2,4,6-TCP	PCP	Time	Dil.Fac.
1	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13:04	1.0
2	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13:26	1.0
3	A-RM 1 mg/l	1.0269	1.0444	1.0273	1.0475	1.0477	1.0280	1.0066	1.0831	1.0060	1.0380	1.0380	13:48	1.0
4	A-RM 20 mg/l	20.6093	20.7577	20.8390	20,7057	20.6421	20.5796	20.6968	20.6619	20.0971	20.4772	20.1433	14:10	1.0
5	"0"	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	14:32	1.0
6	H2O	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	14:54	1.0
7	H2O	n.a.	0.0006	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15:16	1.0
8	H2O+A-RM	0.0863	0.0863	0.0678	0,0884	0.0870	0.0885	0.0882	0.0837	0,0880	0.0875	0.0756	15:38	1.0
9	H2O+A-RM	0.0848	0.0822	0.0575	0,0953	0.1001	0.0970	0.0999	0.0890	0,1035	0.1041	0.0839	16:00	1.0
10	STOP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:18	1.0

TABLE 23.Results obtained from analysis of different matrixes

No.	Name	P	4-NP	2,4-DNP	2-CP	2-NP	2,4-DMP	4-C-3-MP	2-M-4,6-DNP	2,4-DCP	2,4,6-TCP	PCP	Time	Dil.Fac.
1	acetonitryl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	15:44	1.0
2	A-RM 1 mg/l	1.0386	1.0552	1.0656	1.0294	1.0485	1.0389	1.0488	1.1030	0.9645	1.0200	0.9977	16:06	1.0
3	A-RM 20 mg/l	18.5380	18.7411	18.7692	18.5664	18.4884	18.5356	18.6715	18.9484	18.7759	18.4524	18.2920	16:28	1.0
4	"0"	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16:50	1.0
5	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	17:12	1.0
6	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	17:34	1.0
7	1+ARM	0.0850	0.0900	0.0956	0.0905	0.0902	0.0885	0.0900	0.0920	0.0847	0.0893	0.0762	17:55	1.0
8	1+ARM	0.0920	0.0959	0.1000	0.0965	0.0938	0.0939	0.0947	0.0950	0.0937	0.0918	0.0757	18:17	1.0
9	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	18:39	1.0
10	2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	19:01	1.0
11	2+ARM	0.0789	0.0830	0.0882	0.0850	0.0833	0.0823	0.0846	0.0881	0.0855	0.0868	0.0829	19:23	1.0
12	2+ARM	0.0690	0.0742	0.0816	0.0801	0.0776	0.0747	0.0780	0.0833	0.0763	0.0840	0.0853	19:45	1.0
13	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	20:07	1.0
14	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	20:29	1.0
15	3+ARM	0.0756	0.0804	0.0907	0.0834	0.0880	0.0824	0.0851	0.0929	0.0806	0.0909	0.0834	20:51	1.0
16	3+ARM	0.0926	0.0940	0.0937	0.0919	0.0891	0.0915	0.0919	0.0884	0.0862	0.0884	0.0739	21:13	1.0
17	4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	21:35	1.0
18	4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	21:57	1.0
19	4+ARM	0.0890	0.0902	0.0857	0.0882	0.0884	0.0893	0.0901	0.0872	0.0849	0.0884	0.0787	22:19	1.0
20	4+ARM	0.0891	0.0900	0.0885	0.0909	0.0885	0.0897	0.0901	0.0884	0.0897	0.0881	0.0783	22:41	1.0
21	5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23:03	1.0
22	5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	23:25	1.0
23	5+ARM	0.0904	0.0941	0.0943	0.0938	0.0931	0.0928	0.0940	0.0952	0.0945	0.0941	0.0869	23:47	1.0
24	5+ARM	0.0894	0.0933	0.0915	0.0919	0.0923	0.0921	0.0930	0.0931	0.0884	0.0938	0.0868	00:08	1.0
25	5+ARM	0.0948	0.0978	0.0993	0.0977	0.0964	0.0960	0.0970	0.0966	0.0929	0.0962	0.0837	00:30	1.0
26	5+ARM	0.0951	0.0986	0.0986	0.0974	0.0981	0.0967	0.0982	0.0987	0.0938	0.0978	0.0856	00:52	1.0
27	STOP	0.0082	0.0007	0.0006	0.0039	n.a.	0.0018	0.0137	n.a.	0.0068	0.0070	n.a.	01:54	1.0

Appendix 1/7

TABLE 24. Recoveries from 11 phenols obtained from analyzing different matrixes in percentage (%F)

Compound	Recovery (%f)
Phenol	86.02
4-nitrophenol	88.55
2,4-dinitrophenol	87.27
2-chlorophenol	90.44
2-nitrophenol	90.04
2,4-dimethylphenol	89.21
4-chloro-3-methylphenol	90.65
2-methyl-4,6-dinitrophenol	90.28
2,4-dichlorophenol	88.32
2,4,6-trichlorophenol	91.11
Pentachlorophenol	80.27

Table 22 and 23 presents the results obtained from different matrixes. Table 24 shows the recoveries of phenols which should be 80-110% proving the method suitable for analyzing different matrixes. It can be concluded that phenols are not present in lake water but the validated method is suitable for analyzing different matrixes. Samples were collected from five different lakes and the sample was analyzed twice for accuracy which is the samples indicated with numbers in the table while samples with reference material were used to check recovery (1+ARM). Pure acetonitryl was used to wash the HPLC equipment, column to prevent interferences of results obtained before running the sequence of the sample.